EFFECTS OF DIPHENYLHYDANTOIN ON SYNAPTIC TRANSMISSION

by

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I. GENERAL INTRODUCTION

Diphenylhydantoin (Dilantin) is firmly established as a clinically effective anticonvulsant drug. Elucidation of the nature of the action of Dilantin against chemically and electrically induced seizures in laboratory animals has been the object of numerous investigations since its introduction by Putnam and Merritt in 1937. Whereas the gross effects of Dilantin have been characterized as a result of such studies, the discrete effects upon synaptic transmission and the fundamental actions of the drug which underlie the anticonvulsant action remain largely unknown.

It was early recognized that Dilantin, unlike older antiepiletpic agents (bromide and phenobarbital), has anticonvulsant properties clearly distinguishable from sedative effects (Putnam and Merritt, 1941). Thus, while a given drug may exhibit both effects, it is evident that the two are separable. Indeed, other effective anticonvulsants, e.g., trimethadione and phenacemide, are essentially devoid of sedative actions. Although the mechanisms by which sedation and anesthesia are produced are not fully understood, it appears that the majority of local and general anesthetics, including barbiturates, produce anesthesia through stabilization of the neuronal membrane (Butler, 1950). It seems likely that stabilization is achieved by a variety of agents through basically similar actions on neuronal membranes. Likewise, it is not unreasonable to suppose that the

common denominator of the actions of anticonvulsant drugs is an effect upon neuronal processes distinct from that of membrane stabilization.

Elucidation of the type and fundamental mechanism of action of a given anticonvulsant drug necessarily involves a multifaceted research program. It would appear, however, that most of the desired information could be obtained from investigating four separate phases: (1) effects of the agent upon properties of axons, such as threshold, post-stimulation refractoriness and repetitive firing; (2) effects upon transmission in simple synaptic systems, including facilitation, inhibition, post-tetanic potentiation and response to repetitive stimulation; (3) effects upon transmission in complex cortical and sub-cortical synaptic systems, including effects upon induced seizure activity; and (4) effects of the agent upon such fundamental neuronal processes as membrane ionic fluxes and neuronal metabolism.

Dilantin offers several advantages as a candidate for study. Firstly, its clinical effectiveness is well established and its anticonvulsant properties have been extensively investigated. Secondly, Dilantin is effective against maximal convulsive activity (grand mal) in doses which produce no sedation or other pronounced effect upon the nervous system. Since the effects of Dilantin upon impulse transmission in peripheral nerve have been rather thoroughly studied (Toman, 1949, 1952; Korey, 1951), this present research is concerned with the next phase of the problem, that is, the effects of Dilantin upon transmission in simple synaptic systems. In this investigation, monosynaptic and polysynaptic pathways of the spinal cord and a monosynaptic pathway in the stellate ganglion have been studied.

These synaptic systems were chosen because of their accessibility and because considerable information is available concerning the characteristics of synaptic transmission at these sites.

The purpose of this investigation, therefore, was to determine the principal effects of Dilantin upon properties of transmission in relatively simple synaptic systems. The results are largely descriptive, and it cannot be stated that the observed effects upon transmission are related directly to the anticonvulsant action of Dilantin. However, they provide clues which will be utilized in carrying out contemplated experiments on the effects of Dilantin on more complex suprasegmental synaptic systems.

II. GENERAL METHODS

Cats were used in all experiments. The animals were anesthetized with ether, a tracheal cannula was inserted, and the spinal cord was transected at the atlanto-occipital junction. Artificial respiration was begun, and the anesthesia was discontinued. A minimum of one hour in stellate ganglion experiments and three hours in spinal cord experiments was allowed for elimination of the ether. Decamethonium, in doses of 0.5 mgm./kgm., was employed as needed for paralysis during the surgery. In some spinal cord experiments, d-tubocurarine, in doses of 0.3 mgm./kgm. was used for paralysis. These agents rarely were required during the period of recording. When they were employed, minimal paralytic doses were used, and were observed to be without effect upon synaptic transmission. The temperature of the animal was maintained at the desired level by infra-red heat lamps during surgery and during the period of recording.

Nerves selected for stimulation or recording were dissected free and bathed in heavy mineral oil held in a pool by the surrounding tissue. The nerves were raised into the oil by hook-electrodes made of platinum or silver wire. Monophasic, "square-wave" stimuli were supplied singly, in pairs, or in trains from two synchronized Grass Model S-4 stimulators. In spinal cord experiments, the stimuli were referred to ground; but in stellate ganglion experiments, because of the propinquity of the stimulating and recording electrodes, the stimuli were isolated from ground by two Grass Stimulus Isolation Units. The animal was grounded in common with the shielded cage by a wire to the headholder or to exposed tissue at a point

distant from the stimulating electrodes.

Whenever possible, responses were recorded monophasically by placing one electrode on a cut or crushed portion of the nerve. Recorded potentials were amplified through Grass Model P-4 Pre-Amplifiers (two for dual-channel recording). Push-pull input was always employed, but the output of the pre-amplifier was referred to ground and fed through a switching panel to the amplifiers of a DuMont Type 322-A Dual-Beam Cathode-Ray Oscillograph. All amplifiers were capacity-coupled, and the frequency response was determined by filters in the pre-amplifier. The half-amplitude band width was 1 to 3,500 cps. for spinal cord experiments and 1 to 900 cps. for stellate ganglion experiments.

Responses displayed on the dual-beam oscillograph were photographed by a Grass Kymograph Camera, Model C4C. A cathode-ray tube wired in parallel to the main tube permitted monitoring of both channels while the camera was in front of the oscillograph. A separate single-beam oscillograph was employed to monitor either channel as desired; potential and time axes were independent of those of the main oscillograph. An audio monitor, consisting of an audio amplifier and loud speaker, was used in most experiments to check synchronization of instruments and continuity of the recording channels and to monitor background activity and interference. A conventional audio oscillator was used for introducing time signals.

Photographic records were taken on 35 mm. Linograph 697 paper. Comonents of the responses on the film were measured either directly or following enlargement (about 4x) in a Leitz ("Valoy") enlarger. Direct measurements were made to the nearest 0.5 mm.; precision was doubled by enlargement.

Where pertinent, these measurements were converted into values for voltage or time by reference to relevant calibration markers in the record.

Dilantin sodium dissolved in 0.9% sodium chloride solution (0.9% saline) was used in all experiments. All injections were made intravenously, usually in the cephalic vein but occasionally in the femoral vein. In some of the early experiments, Dilantin was used in a concentration of 20 mgm./ml., brought in to solution by addition of a small amount of sodium hydroxide. Injections were made rapidly, and in three spinal cord experiments a transient depression of reflex excitability was noted. In one experiment in which the dose of 30 mgm./kgm. of Dilantin was given in one minute, complete areflexia ensued. Transmission from muscle-nerve to dorsal root was normal, but no reflex responses could be secured with any stimulus. The initial level of reflex excitability later returned. Since rapid intravenous injection of Dilantin produces transient hypotension (Harris and Kokernot, 1950; van Harreveld and Feigen, 1950; van Harreveld et al., 1951), and since a rise in flexor reflex threshold accompanies a fall in blood pressure (Pedersen, 1954), it was felt that the transient areflexia following Dilantin might be secondary to a fall in blood pressure and not due to a direct action of Dilantin on neurons. Accordingly, blood pressure records were made on a spinal cat while Dilantin, in a concentration of 2 mgm./ml., was injected intravenously at various rates. First a solution of sodium hydroxide in 0.9% saline, with the same titratable alkalinity as that of the Dilantin solution, was injected rapidly. Six minutes were taken to inject 45 ml., a volume equivalent to that containing a 30 mgm./kgm. dose of Dilantin. No fall in blood pressure was observed; on the contrary, the

pressure rose from 55 to 70 mm. Hg and became stabilized at the higher level. Rapid injection (10 seconds) of 2 mgm./kgm. of Dilantin produced a transient fall in blood pressure of 20 mm. Hg. Rapid injection of 6 mgm./kgm. produced periodic asystole and a fall in pressure of 30 mm. Hg. When heart rate and blood pressure had returned to normal, the remainder of the 40 mgm./kgm. dose of Dilantin was injected at the rate of 0.5 mgm./kgm./minute and only a slight fall in blood pressure occurred. In a second spinal animal it was found that Dilantin could be administered intravenously at the rate of 1 mgm./kgm./minute without producing marked changes in either the blood pressure or the electrocardiogram (cf. Scherf, 1943). Therefore, unless stated otherwise, the concentration of Dilantin was 2 mgm./ml., and the rate of administration did not exceed 1 mgm./kgm. minute.

In this study, intravenous doses of Dilantin from 10 to 40 mgm./kgm. were used. These values were found by Toman et al. (1946) to define respectively the minimally effective anticonvulsant and the minimally neurotoxic doses of Dilantin given to cats by the intraperitoneal route. Similar studies after intravenous administration have not been made. However, it is probable that intravenous doses of Dilantin employed in this investigation are generally comparable to similar intraperitoneal doses, since the absorption of injected Dilantin is rapid (Swinyard et al., 1953), and the experiments were of several hours duration. Destruction or excretion of Dilantin could be safely disregarded in these acute experiments since in cats the anticonvulsant effect of Dilantin persists for many days after a single protective dose (Toman et al., 1946).

III. EFFECTS OF DIPHENYLHYDANTOIN ON SYNAPTIC TRANSMISSION IN THE SPINAL CORD

A. Introduction

The ventral root response to stimulation of a homolateral dorsal root of the same or a neighboring segment consists of a sharp initial spike of approximately 1 msec. duration followed by a series of irregular discharges. The first spike was shown by Renshaw (1940) to represent the discharge of motoneurons activated directly by primary afferent fibers. The later spikes represent activity in the polysynaptic pathways; the duration of the discharge depends upon the intensity of the dorsal root stimulus (Lloyd, 1943a). The afferent neurons which synapse directly with motoneurons arise exclusively in the muscle spindles. These are the largest fibers (Group Ia) of the dorsal root and hence have the lowest threshold (Hunt, 1954). Stimulation of the large muscle-nerve afferents, therefore, results in a monosynaptic (2N) reflex response; stimulation of cutaneous-nerve afferents produces only polysynaptic reflex discharges (Lloyd, 1943a,b,c, 1944, 1949b; Hunt, 1954).

Since the monosynaptic pathways of the spinal cord can be activated essentially in isolation, they represent one of the most accessible, simple synaptic systems in the central nervous system; hence transmission in these pathways has been studied extensively. Orthodromic activation of the group Ia afferents results in motoneuron discharge after a central delay of about 0.5 msec. (Renshaw, 1940; Lloyd, 1944). Prolonged synaptic depression follows transmission of an impulse, and recovery, as tested orthodromically, is not

complete for 3 to 4 seconds (Eccles and Rall, 1951a; Jefferson and Schlapp, 1953). Most of this depression is due to processes occurring in the afferent terminals (Brooks et al., 1950), since recovery of motoneurons following antidromic activation is essentially complete within 100 msec. (Lloyd, 1951).

A variety of facilitatory and inhibitory influences can be impressed upon motoneurons and detected by orthodromic monosynaptic testing. Facilitation obtains between motoneuron pools of synergic muscles, and mutually inhibitory influences are exerted between motoneurons of antagonistic muscles. These influences are exerted via collaterals of the group Ia afferents and are confined to the motoneurons of a given myotatic unit (Lloyd, 1949b). The neuron pathways which mediate these facilitatory and inhibitory influences are shown schematically in figure 1. The facilitatory influence decays exponentially and disappears in about 15 msec. after arrival of the impulses at the motoneurons (Lloyd, 1946a, b, 1949b). Direct inhibition, though opposite in direction, is similar in time-course (Lloyd, 1946a, b, 1949b) but has an initial more rapid decay (Bradley et al., 1953). In addition homosynaptic facilitation, similar in mechanism and time-course to facilitation of synergic motoneurons, is observed when conditioning and test stimuli are applied to the same afferent neurons (Lloyd, 1943a; Brooks and Eccles, 1948). More complex patterns of facilitation and inhibition include disynaptic reflex linkages, and influences mediated through polysynaptic pathways. Disynaptic influences are generally opposite in direction to facilitation and inhibition through 2N pathways, though the effects, particularly inhibition, extent to muscles outside of a given myotatic unit (Laporte, 1953). Excitation of polysynaptic pathways creates background activity which is

predominately facilitatory to flexor and inhibitory to extensor motoneurons (Creed et al., 1932; Lloyd, 1944, 1949b; Hagbarth, 1952).

A phenomenon of prolonged potentiation of monosynaptic reflexes following tetanic stimulation of afferent fibers has recently been demonstrated by Lloyd (1949a). Potentiation (post-tetanic potentiation, PTP) of test responses reaches a maximum about 15 sec. after tetanus and declines slowly to disappear after approximately 3 minutes. Jefferson and Benson (1953), in a careful study involving over 30 cats, have shown that there is a definite upper limit to the number of motoneurons discharged during maximum potentiation produced by tetanization of a given muscle-nerve. In most instances the maximum potentiated discharge zone approximates the size of the motoneuron pool of the tetanized nerve. While the potentiated discharge was sometimes observed to be either larger or smaller than that due to the anatomically discrete motoneuron pool, the size of the discharge zone during PTP was found to be quite constant in a given preparation. This technic, therefore, provides a means of measuring the size of the relevant motoneuron pool, a parameter of prime importance in studying variations in levels of excitability in monosynaptic pathways, whether induced by drugs or by other means. Consideration of the significance and mechanism of post-tetanic potentiation is reserved for a later section.

Polysynaptic pathways of the spinal cord are very complex, since their excitation produces activity in widely separated neurons in both halves of the cord. In this study only homolateral polysynaptic responses were considered. These responses were recorded from a single ventral root and were induced either by stimulation of the corresponding dorsal root or of a

cutaneous nerve. Because of the complexity of these pathways and because of the difficulty in obtaining meaningful measurements from the irregular discharge, studies of facilitation and inhibition have been made only in monosyanptic pathways. Post-tetanic potentiation in polysynaptic pathways has been little studied. Although Lloyd (1949a) believed PTP to be a phenomenon occurring at most synapses, he failed to demonstrate it for polysynaptic pathways. In this investigation, the effects of Dilantin on interneurons have been considered because of the importance of intermuncial background activity in influencing the excitability of 2N pathways and because certain drugs, e.g., mephenesin, are said selectively to depress transmission in polysynaptic pathways (Kaada, 1950; King and Unna, 1954; Wright, 1954; but cf. Latimer, 1955).

B. Methods

The general technics and the instruments employed in this investigation have been described above. Following transection of the cord, a laminectomy was performed in the lumbo-sacral region. The dorsal portions of the vertebrae were removed for about 6 segments, extending as far caudally as the second sacral roots. The dura was not opened until the animal was rigidly fixed and all peripheral nerves to be used were exposed and covered with mineral oil. The dura was opened by a midline incision extending the full length of the cord exposure, and the cerebrospinal fluid was removed and replaced with warm mineral oil. Slight elevation of the cord was achieved by pinning the dura back over the rim of the cut vertebrae. Separation and section of appropriate spinal roots were then performed. The muscle-nerves employed for stimulation were those to the two heads of gastrocnemius

muscle and to tibialis anterior. To prevent antidromic activation of motoneurons, therefore, the relevant homolateral ventral roots (L_7, S_1 and S_2) were severed (Reighard and Jennings, 1949; Jefferson and Benson, 1953). These roots were also cut when the sural nerve, a purely cutaneous nerve, was to be stimulated. In dorsal root-ventral root (DR - VR) preparations, intact afferent inflow was not necessary; hence both dorsal and ventral roots of the segments mentioned were cut on both sides. In these studies all reflex responses were recorded from S_1VR. All peripheral nerves used for stimulation were severed distally. Reflex muscle contraction from peripheral nerve stimulation was usually absent or minimal, so that denervation of other muscles rarely was necessary.

One of the most important variables affecting the level of reflex excitability in preparations of this type is the temperature of the spinal cord (Koizumi, Malcolm and Brooks, 1954).[1] Decreasing the temperature of the cord from 38 C to 25 C causes a progressive increase in reflex excitability; decreased excitability results when temperature is lowered below 25 C or raised above 38 C. The increased excitability as temperature is lowered is due to greater invasion of the motoneuron pools by an afferent volley. At 38 C it has been observed (in practice preparations prior to this study) that a stimulus maximal for group Ia muscle afferents often evokes little or no reflex discharge. Lowering of reflex threshold by decreasing the temperature causes an increase in both monosynaptic and

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[1] The author is grateful to Dr. Brooks for a personal communication regarding the effects of temperature on reflex excitability. Because of this information, the importance of temperature control in determining amplitude and purity of the 2N response was appreciated from the beginning of this investigation.

polysynaptic reflexes, but with stimuli subliminal for the small muscle-afferents a monosynaptic spike can be obtained with very little polysynaptic discharge. In this study, therefore, the cats were maintained at subnormal temperatures during the period of recording. Temperature was maintained by a battery of manually operated heat lamps, arranged so as to warm the animal uniformly. Cooling, when necessary, was done with ice packs. Rectal temperature, as well as the temperature of the cord and peripheral nerve oil pools, was checked frequently, and the variation in temperature in a given experiment was kept within 1 C during the recording period. Unless stated otherwise, the animals temperature was held at 32 C.

C. Experimental Results

The effects of Dilantin upon transmission of isolated impulses in monosynaptic and polysynaptic pathways of the spinal cord are illustrated by the responses shown in figure 2. In assigning slight changes in response to an effect of the drug, inherent variability of the response must be considered. Further, since administration of Dilantin requires some time, the stability of the preparation is very important. In the DR-VR experiments (figure 2 A and B), the preparations were very stable during the control period, which lasted several hours, and during the periods of testing following administration of Dilantin. In experiment S-12 (figure 2 A), Dilantin was injected at the rate of 1 mgm./kgm./minute and the responses shown were obtained during the injection when the indicated amounts had been given. Note the slight reduction in monosynaptic spike and the more marked reduction in disynaptic and polysynaptic activity. In another DR-VR experiment, the effect of Dilantin on polysynaptic activity was less pronounced.

In experiment S-11 (figure 2 B), Dilantin was given in 10 mgm./kgm. increments with about 1 hour of testing after each dose. The reductions in monosynaptic spike height were observed immediately after injection of the drug and are not due to a slow decrease in level of excitability. Control responses show little activity in polysynaptic pathways in this experiment, though the single major spike may contain both monosynaptic and disynaptic discharges. Monosynaptic responses elicited by muscle-nerve stimulation are quite variable, and slight changes in reflex excitability greatly affect the spike height. This is because of the small number of motoneurons responding to each stimulus (Jefferson and Benson, 1953; Jefferson and Schlapp, 1953). Small changes in response produced by Dilantin, therefore, could easily be obscured by changes in the level of reflex excitability, and the aim in such experiments has been to maintain the monosynaptic spike as nearly constant as possible, rather than to determine effects of the drug upon it. In experiment S-10 (figure 2 C), the monosynaptic spike was maintained essentially constant, but Dilantin reduced the amount of polysynaptic discharge. The polysynaptic activity in this case was much greater than that ordinarily seen with muscle-nerve stimulation, because the stimulus intensity was increased to excite the small afferent fibers of the muscle-nerve (Lloyd, 1943a; Hunt, 1954).

Since motoneurons discharged through monosynaptic pathways are refractory to stimulation by impulses arriving later through polysynaptic pathways (Lloyd, 1951), a decrease in polysynaptic discharge characteristically follows an increase in the monosynaptic spike. The reduction in polysynaptic activity observed after Dilantin cannot be explained on this basis, however,

because the monosynaptic spike was decreased by the drug. Furthermore, the pure polysynaptic discharge produced by stimulation of the sural nerve was reduced by Dilantin (figure 2, D). In this experiment, a dose of 10 mgm./kgm. reduced the polysynaptic discharge by approximately 50% (note gain change). In another similar experiment the effect of Dilantin was less pronounced, a reduction of 50% occurring only after 30 mgm./kgm. of the drug.

Effects of Dilantin on primary facilitation between the motoneuron pools of the two heads of gastrocnemius are shown in figure 3. The curves obtained are typical (Lloyd, 1946) and fail to suggest an effect of Dilantin (30 mgm./kgm.) on facilitation. This is the only experiment in which facilitation between synergic muscles has been studied. However, homosynaptic facilitation, observed when two stimuli are applied to the same muscle-nerve or dorsal root, has been studied in several experiments. Dilantin, in doses up to 30 mgm./kgm., has never been observed to affect this facilitation. In fact, in experiment S-11 the maximum discharge during homosynaptic facilitation was observed to be 100% of the estimated relevant total subliminal fringe (maximum potentiated discharge minus control discharge, cf. figure 9) both before and after 30 mgm./kgm. of Dilantin. There is likewise no indication of an effect of Dilantin on primary inhibition (figure 4). The curves shown here differ from those obtained for pure monosynaptic inhibition (Lloyd, 1946a, 1949b; Bradley et al., 1953) in that early facilitation (at about 4 msec.) is present. This facilitation is mediated through disynaptic pathways (Laporte, 1953) and commonly contaminates primary inhibition because of the slight difference in threshold

between the group Ia afferents from muscle spindles and the Ib afferents from tendon organs concerned with disynaptic reflexes (Bradley and Eccels, 1953; Bradley et al., 1953; Hunt, 1954). Conditioning and test responses and some inhibited responses are shown in figure 13A.

Synaptic depression following transmission of a single impulse in monosynaptic pathways has been studied in several experiments. Curves similar in shape to those of Eccles and Rall (1951a) have been obtained before and after administration of Dilantin. The degree of depression observed at a given time after monosynaptic activation depends to a great extent upon the relative numbers of neurons in the discharge zone and in the subliminal fringe. It has been difficult to maintain the discharge zone (to muscle-nerve stimulation) constant over the time required for these studies, and consequently the results obtained with pure monosynaptic responses have failed clearly to show an effect of Dilantin on synaptic depression. DR-VR preparations possess greater stability, and it was necessary to study synaptic depression in this type of preparation in spite of the unavoidable influence of polysynaptic activity (Lloyd, 1943a, 1944). The effect of Dilantin on synaptic depression, as indicated by the monosynaptic response, is illustrated in figure 5. This effect of Dilantin to deepen synaptic depression also manifests itself in increasing fatigue during repetitive stimulation (figure 6). These data were obtained in experiment S-11, in which only slight reduction in monosynaptic spike was produced by Dilantin (see figure 2 B), and little polysynaptic discharge was observed. In another DR-VR preparation (S-12, see figure 2 A), considerable polysynaptic discharge was observed in the control responses, and this discharge was reduced by Dilantin.

Synaptic depression was not studied, but the response to repetitive stimulation was different from that shown in figure 6. In this case fatigue during repetitive stimulation was less after Dilantin than in the control series. Because of the complexity of the influences of background intermuncial activity on motoneurons (Renshaw, 1942; Bernhard, 1952), it cannot be stated whether the decrease in fatigue resulted from depression of polysynaptic activity by Dilantin. However, the results obtained in the experiment in which little polysynaptic activity was initially present would appear to be more meaningful.

The most striking effect of Dilantin upon activity in monosynaptic pathways is reduction of post-tetanic potentiation (PTP). This effect was observed in the first experiment and has been studied in nine preparations. In all cases the maximum potentiated discharge was reduced by Dilantin. In considering PTP, attention has been paid only to the size of the maximum potentiated discharge. Measurement of the change in spike height produced by a tetanus is not particularly meaningful; the maximum potentiated discharge zone remains essentially constant (Jefferson and Benson, 1953), hence the ratio of potentiated spike height to control spike height varies inversely with the level of reflex excitability at rest. In one experiment PTP ratio dropped from about 10 to 2 as the control spike progressively increased, but the height of the potentiated spike remained approximately the same. It is probably that at very low levels of excitability PTP ratio would vary in the same direction as control spike height because of inability to discharge all relevant neurons following a maximal tetanus. It does not appear, however, that this is an important consideration in this study. Examination of the

data presented on PTP attests to the validity of disregarding the effects of Dilantin on PTP ratio; Dilantin generally reduces monosynaptic spike height, but the reduction in the maximum potentiated discharge is disproportionately greater. Further, in some experiments (cf. figure 9, curves d and e; figure 10; figure 11 B), changes in control and potentiated spike were in opposite directions.

The stability of PTP with time is illustrated in figure 7. These two control determinations were made 3.5 hours apart, with other monosynaptic testing being carried out during much of the interval.

Effects of Dilantin on PTP induced by muscle-nerve stimulation are shown in figure 8, and by dorsal root stimulation in figure 9. Curves a, b, c, and d of figure 9 were determined in succession over a period of 3 hours. Curve e, however, was determined 14 hours after curve d and the data of figure 10 were obtained in the meantime. To observe the effect which followed an increase in the dose of Dilantin from 30 mgm./kgm. to 40 mgm./kgm., therefore, compare curve c of figure 10 and curve e of figure 9. The progressive decrease in PTP observed with time after 30 mgm./kgm. of Dilantin (figure 10) suggests that some time is required for maximum effectiveness of this agent by the intravenous route. Though this experiment was continued longer than any control experiment, there was nothing to suggest that the preparation was not in excellent condition. Another preparation (S-1) was carried in good condition for 30 hours after administration of Dilantin. PTP was determined less precisely by direct observation of the oscilloscope; but, after initial reduction by Dilantin, PTP appeared to remain essentially constant.

In figure 11 are shown the effects of tetanic stimulation for 15 seconds at varying frequencies. Note in the control series that stimulation at 300/sec. and 500/sec. produces essentially the same effect. This was observed by Lloyd (1949), and Jefferson and Benson (1953) have shown this maximum discharge to be the total relevant motoneuron pool. That these two frequencies have almost identical effect after Dilantin suggests that a new maximum is approached, and this limit is clearly less than the relevant motoneuron pool. The data also suggest that Dilantin is more effective in reducing PTP at low frequencies than at high frequencies.

Invariably it has been observed that as potentiation subsides the potentiated discharge falls to the level of the control discharge and remains there. This is true for PTP before and after administration of Dilantin. However, all the data presented have been obtained with supramaximal tetanic and test shocks. In two experiments submaximal test shocks have been employed before and after tetanization with supramaximal stimuli. In both cases PTP in the control determination was nearly maximal and followed the same time-course as PTP with supramaximal test shocks. After Dilantin the discharge secured by submaximal post-tetanic test shocks was much less than that obtained with supramaximal stimuli, and about one minute after tetanus the potentiation passed into depression lasting several minutes. These results are suggestive of an interesting effect of Dilantin; however, wider variation in the intensity of the test shocks must be employed to relate quantitatively the potentiated discharge and degree of depression to the intensity of the test stimuli.

In 1943, Lloyd determined the relation between the size of presynaptic

volleys and postsynaptic 2N discharges in a DR-VR preparation. A plot of these two variables gave a sigmoid curve (Lloyd, 1943a, fig. 1). The relationship between these two variables does not appear to have been determined during the period of post-tetanic potentiation; such information would seem to be particularly significant in view of the observations of Jefferson and Benson (1953). The relations between input and output in the monosynaptic pathway at rest were determined in two experiments (S-11, S-12). The two curves were practically identical in spite of the great difference in polysynaptic activity in the two preparations (cf. fig. 2 A and B). In one experiment (S-12), input-output relations were determined during PTP by testing during the period from 8-40 seconds after the tetanus, when PTP is nearly maximal. Curves obtained at rest and during PTP in the same experiment are shown in figure 12. Observe that the maximum value for the 2N spike is taken to be the maximum potentiated discharge (assumed total relevant motoneuron pool), and the maximum discharge at rest is only 37% of this value. Choice of the maximum dorsal root spike (abscissa) is somewhat arbitrary; only the largest afferents end directly on motoneurons, hence excitation of other fibers increases the dorsal root spike without affecting 2N reflex discharge. Because of overlapping thresholds it is impossible to excite all of the relevant afferent fibers (group Ia) without also exciting a large fraction of group Ib and some group II fibers (Lloyd, 1943a; Bradley and Eccles, 1953; Hunt, 1954). Observe from the records of figure 13 D that a large disynaptic spike and some polysynaptic discharge occurs before the monosynaptic spike is maximal. These considerations apply to absolute values of the abscissa, but do not affect comparisons between the

two curves since the same range of stimulus intensities was employed in both determinations.

To appreciate the effect of PTP, compare the 2N reflex discharge at rest and during PTP for several points on the abscissa. In figure 13 D, record 1 shows a dorsal root volley which at rest produces no 2N discharge, but record 3 indicates that during PTP a smaller dorsal root volley produces a large 2N spike (note change in gain of lower trace). The curves in the lower portion of figure 12 show the distributions of synaptic thresholds, in terms of number of afferent fibers activated, for synapses in the 2N pathway at rest and during PTP. Observe that PTP decreases the mean threshold, decreases dispersion about the mean and increases the size of the population by including motoneurons which could not be activated at rest by a maximal afferent volley. The areas under the curves are proportional to the respective maximal discharge zones.

The effects of Dilantin on activity in polysynaptic pathways have been studied in several experiments. However, except for the effects upon transmission of isolated impulses, the results are incomplete and cannot briefly be summarized. An attempt has been made to study the effects of Dilantin on PTP in polysynaptic pathways. This phenomenon is not easily demonstrated (Lloyd, 1949), although Downman et al. (1953) have observed it is some instances (cf. also Eccles, 1953). The records in figure 13 E, obtained in a control experiment, show definite potentiation of polysynaptic discharge to sural nerve stimulation following a tetanus of 75 stimuli per second for 5 seconds. The results to date suggest that the parameters of tetanic stimulation for maximum potentiation are very different for monosynaptic

and polysynaptic discharges.

In many experiments, at the conclusion of testing the effects of Dilantin, other drugs, most frequently strychnine or pentylenetetrazol, have been administered in an attempt to study modifications of the effects of Dilantin by these agents. No conclusions are warranted from the data presently available.

IV. EFFECTS OF DIPHENYLHYDANTOIN ON TRANSMISSION IN THE STELLATE GANGLION

A. Introduction

The stellate ganglion has been included in the study of the effects of Dilantin on synaptic transmission because it is a simple, accessible synaptic system. There is neither anatomical (de Castro, 1951) nor physiological (e.g., Eccles, 1953) evidence for interneurons in sympathetic ganglia; hence effects exerted at these sites are more easily interpreted than effects upon the monosynaptic pathways of the spinal cord. Interpretation of the results is further simplified by the absence of specific inhibitory fibers in the stellate ganglion (Eccles, 1953; Job and Lundberg, 1953). In addition, the transmitter agent at autonomic ganglia has been identified, and considerable information is available relating electrical and chemical events during transmission under a variety of conditions (Rosenblueth, 1950; Eccles, 1953; Fatt, 1954). With the exception of inhibition, all aspects of synaptic transmission studied in the spinal cord can be investigated in the stellate ganglion, and this permits comparison of the effects of Dilantin on transmission in two synaptic systems which differ in certain anatomicaly and functional respects.

B. Methods

Following transection of the spinal cord and the introduction of artificial respiration, the thoracic cavity was opened on the left side. The ventral half of ribs 2 through 6 were removed on the left side and the lung was retracted away from the stellate ganglion. Blunt dissection was employed to free the preganglionic sympathetic trunk (down to thoracic ramus

IV) and the inferior cardiac nerve for about 3 cm. from the ganglion. The tissue immediately surrounding the ganglion was not disturbed. The inferior cardiac nerve was cut distally, ramus III was severed, and the sympathetic trunk was cut just proximal to ramus IV. The electrodes were placed as indicated in figure 14. The proximal recording electrode was about 1 to 1.5 cm. from the ganglion and the interelectrode distance was about 1 cm. Recorded postganglionic responses were rendered monophasic by crushing the nerve over the distal electrode.

In control studies, variations in temperature from 30 to 37 C were found to have little effect upon transmission in the stellate ganglion. Therefore, temperature was not controlled as critically as in spinal cord experiments. Rectal temperature was maintained at about 35 C during the period of recording.

With the electrode positions used in this study (cf. figure 14), none of the fibers which were stimulated passed through the stellate ganglion without synapsing. In addition, the relevant preganglionic fibers are mainly B fibers (Eccles, 1953); hence the recorded postganglionic response consists of one major spike (cf. figure 15). Occasionally the response included a later, smaller spike (cf. Job and Lundberg, 1953), due presumably to stimulation of preganglionic C fibers (cf. Eccles, 1952b). It was usually possible to make the initial spike maximal without significant contamination by the later spike. Stimuli in all experiments were supramaximal for the initial spike; measurements of the smaller spike have not been made.

C. Experimental Results

The effects of Dilantin on transmission of isolated impulse are illustrated by the records of figure 15 A. In this experiment spike height

was reduced 25% by 30 mgm./kgm. of Dilantin. This reduction was greater than that ordinarily observed, and in some experiments (cf. figures 17, 18 and 20) the postganglionic spike was little affected by this dose of the drug. The constancy of the response during testing before and after administration of Dilantin indicates that the reduction in spike height in this instance was not due to instability of the preparation. An increase in response latency following Dilantin will also be noted in the records of figure 15 A. The total latency was 18 msec. for the control responses, and Dilantin caused an increase in latency of about 6 msec. In general, latency bears an inverse relation to the number of postganglionic neurons responding; in those experiments in which there was little or no change in spike height with Dilantin, latency was also little affected. Total latency has not been resolved into its various components.

The records in figure 15 B illustrate the changes in response form during PTP. The slow negative deflection following the spike in records 2 and 3 is the postsynaptic potential (Eccles, 1952 a, b; Job and Lundberg, 1953), and presumably represents prolonged transmitter action (Eccles, 1953; Fatt, 1954). This potential is not an active response in the postganglionic nerve but is conducted electrotonically from the ganglion cells. It is not normally observed under the recording conditions employed, and its presence during PTP signifies excessive depolarization following transmission of each test impulse.

Heterosynaptic facilitation has not been investigated but homosynaptic facilitation, observed when conditioning and test stimuli are applied to the same preganglionic fibers, has been studied in several experiments. Results

are most meaningful when facilitation is compared in situations where the subliminal fringe is constant. It can be assumed that the subliminal fringe remained constant in two experiments (G-17 and G-18) since Dilantin did not reduce the discharge zone. No effect of Dilantin upon homosynaptic facilitation was observed in either experiment.

At short intervals between homosynaptic conditioning and test stimuli, facilitation predominates; but at longer intervals depression becomes prominent and is the predominant effect after 20-50 msec. (Job and Lundberg, 1953). The effects of Dilantin on depression in three experiments are shown in figure 16. In each instance it appears that Dilantin deepens synaptic depression following transmission of a single impulse. This effect also manifests itself during repetitive activation of the synapse. Figure 17 shows the effects of Dilantin on the response to repetitive stimulation at three different frequencies. These results were obtained in a single experiment, but repetitive stimulation has been studied in 4 other experiments over a frequency range of 10 to 75 stimuli/second. The curves in figure 17 are representative of the data obtained in those experiments in which Dilantin had little effect upon the discharge zone. In the two experiments in which Dilantin decreased the discharge zone, and thereby increased the subliminal fringe, a slightly different pattern was observed. Though the Dilantin curves began at a lower level, there was a tendency in many cases for them to climb toward the control curves during the early period of stimulation. This pattern can be explained by increased facilitation resulting from a greater subliminal fringe. Even in these experiments clear evidence of increased fatigue following Dilantin was obtained at some stimulus frequencies.

Post-tetanic potentiation was studied in 4 experiments in which the stimulating electrodes were between rami II and III. Clear evidence of reduction in PTP by Dilantin was not obtained, though in some experiments there was suggestion of slight reduction. Beginning with experiment G-18, the stimulating electrodes were placed between rami III and IV. With this electrode placement, fewer preganglionic fibers were stimulated and hence the subliminal fringe was increased (cf. Larrabee and Bronk, 1947). Dilantin was observed to have a pronounced effect upon PTP in this experiment as well as in another experiment (G-20) in which this electrode placement was used (figure 19).

The postsynaptic potential (p.s.p.) has not been carefully studied, nor have any after-potentials been measured, because only capacity-coupled amplifiers were available for this investigation. However, measurements of the p.s.p. during PTP have shown it consistently to be reduced by Dilantin, and most strikingly (figure 20) in those experiments (G-18, G-20) in which the stimulating electrodes were placed between rami III and IV.

Figure 20 contains portions of two records from experiment G-18 illustrating the effect of Dilantin on PTP and on the p.s.p. Note also changes in the pattern of the response during tetanization. During the tetanus after Dilantin, early fatigue is evident, and the response falls almost to zero (cf. figure 17). However, this is followed by an apparent recovery of transmission. While this increase in response height is not explained, it has been noted in several records, obtained during high frequency stimulation following Dilantin, that the postganglionic response tends to be asynchronous with the stimuli. Frequently large spikes and small spikes appeared

alternately. Because of the low paper speed it cannot be stated whether this occurred in the record shown.

Since Dilantin hastens transmission failure during repetitive stimulation, it might be argued that the reduction in PTP produced by the drug is a consequence of a less effective tetanus. However, from present evidence regarding the mechanism of PTP, precisely the opposite effect would be expected. Larrabee and Bronk (1947) have shown that antidromic tetanization produces depression rather than potentiation to orthodromic testing. In the spinal cord, orthodromic tetanization produces depression of the postsynaptic unit which must be overcome by potentiation referable to changes in the presynaptic terminals (Lloyd, 1949; Eccles and Rall, 1951a, b). Further, tetraethylammonium enhances transmission failure during repetitive stimulation, but increases the efficiency of a given tetanus, presumably by limiting postsynaptic discharge (Esplin et al., 1955). Thus, reduction of PTP by Dilantin does not appear to be a consequence of reduced activity in the postsynaptic units during the tetanus.

The experiments described have been performed in preparations which were stable and in good condition at the conclusion of testing. The recording period was never longer than 4 hours, yet in this and in other studies (Esplin et al., 1955) such preparations have been maintained for longer than 8 hours. It was felt that the change in pattern during repetitive stimulation (experiment G-6, figure 17) might be due to depletion in stores of mediator, although from the data of Perry (1953) a decrease in acetylcholine output of only about 5% would be expected even if no synthesis occurred during the experiment. In one control experiment, the change in pattern

during repetitive stimulation before and 30 minutes after death of the animal by asphyxia was less than that shown in figure 17.

V. DISCUSSION

Dilantin appears to exert a greater depressant effect upon the transmission of impulses in polysynaptic pathways than upon impulses in the two-neuron reflex arc. No valid conclusions regarding the locus or mechanism of action of the drug would appear to be implied by this observation, nor would it appear to do justice to other, perhaps fundamentally more important, actions of Dilantin to consider it an 'interneuron depressant' drug.

No evidence of an effect of Dilantin on facilitation in either the spinal cord or stellate ganglion was obtained in this study. Inhibition was not studied in the ganglion and was investigated in only one experiment in the spinal cord. While no effect of Dilantin on inhibition was noted, the dose was only 10 mgm./kgm., and it cannot be concluded that this process is unaffected by the drug.

Since the principal effects of Dilantin on transmission in monosynaptic pathways of the spinal cord and in the stellate ganglion are qualitatively identical, they may be discussed together. Dilantin deepens depression following transmission of a single impulse and hastens fatigue during repetitive stimulation. It is not unlikely that the two effects are manifestations of a single action; enhanced fatigue may be merely summation of enhanced depression. If Dilantin were shown to exert a similar effect at its appropriate cerebral loci of action, it would be difficult to reject such an effect as one component of the anticonvulsant action of the drug.

Post-tetanic potentiation has been reported as occurring at a variety of junctional tissues (Eccles, 1953; Fatt, 1954), and a common mechanism appears to underly PTP at all sites. The most definitive studies have been

on the spinal cord and autonomic ganglia, and all the results indicate that tetanization produces some change in the afferent terminals which is responsible for the prolonged potentiation (Larrabee and Bronk, 1947; Lloyd, 1949a; Eccles and Rall, 1951a, b; Eccles, 1952a, b). Following tetanization, the post synaptic units are depressed to electrical stimulation (spinal cord, Lloyd, 1949a), and senstivity to acetylcholine is not increased (stellate ganglion, Larrabee and Bronk, 1947). Several explanations for PTP have been proposed. Lloyd (1949a) observed a slight increase in intramedullary afferent spike potential which followed the same time course as the potentiated reflex discharge. A causal relationship was suggested for these two potentials; however, Eccles and Rall (1951a) observed potentiation under many circumstances in which no correlation existed between the presynaptic and reflex spikes. Furthermore, in view of the relation between afferent and efferent volleys in the 2N pathway (figure 12; Lloyd, 1943a), it would seem that unique properties of the synapse must be postulated to construct an electrical hypothesis for PTP from such a relationship. Eccles and Rall (1951a) have suggested that activity in the presynaptic terminals alters their spatial relationship with the motoneuron and makes a given afferent impulse a more efficient excitatory agent. No direct experimental evidence has been advanced to support such an hypothesis. While this was initially proposed as the basis for an electrical hypothesis for PTP, Eccles (1953) has now suggested that the excitatory action of a chemical mediator may be enhanced by such spatial alterations. Larrabee and Bronk (1947) proposed, by elimination of other conceivable mechanisms, that a greater amount of mediator is released by each presynaptic volley

during the period of potentiation. Lloyd's suggestion may be rejected, and the two remaining hypotheses are almost indistinguishable since identical effects upon the postsynaptic membrane are predicted, and the different changes in the presynaptic fibers postulated by the two hypotheses are subtle ones and difficult to study by present technics.

It need not be assumed that Dilantin reduces PTP by an action at the site at which processes responsible for PTP occur. However, Dilantin clearly does not reduce PTP by actions on the peripheral afferent or efferent axons (Toman, 1949, 1952). Dilantin reduces PTP while leaving facilitation unaffected. But the postsynaptic unit is common to both processes, and both excitatory and facilitatory influences are apparently produced by the same mediator at a given site (Eccles, 1953; Fatt, 1954). Effects upon the postsynaptic unit must therefore be rejected, since it is difficult to understand how it could respond differently to the same mediator released by different processes. The fine presynaptic extensions of the afferent axons remain, therefore, as the most reasonable site upon which Dilantin acts to reduce PTP, and it is significant that the prolonged depression following transmission of an impulse in 2N pathways of the spinal cord, which is deepened by Dilantin, has also rather convincingly been shown to be due to events occurring in the presynaptic terminals (Brooks et al., 1950; Fatt, 1954).

Dilantin may act to block the potentiating process or to prevent its manifestation once it has occurred. According to the hypothesis of Eccles and Rall (1951a; Eccles, 1953), it is proposed that activity in the presynaptic terminals causes them to swell and 'move closer' to the motoneurons.

If this hypothesis were indeed true, Dilantin would have to decrease presynaptic activity during the tetanus or, alternatively, to 'shrink' the swollen terminals. The former possibility deserves consideration. There is no evidence from peripheral nerve studies to suggest such an action of Dilantin, though admittedly the two situations are not comparable. Also, since the normal process of synaptic excitation is only slightly affected by Dilantin, this conduction block, if it occurs, must be more complete at high frequencies than at low frequencies. PTP, however, is more effectively reduced by Dilantin after low than after high frequency tetanization (figure 11).

The suggestion of Larrabee and Bronk forms perhaps the most plausible basis for consideration of the effect of Dilantin on PTP. It is assumed that through some process, presently obscure, tetanization increases the amount of mediator liberated by a subsequent afferent volley. There is abundant evidence that in normal processes of excitation afferent impulses may die in presynaptic terminals and fail to exert their excitatory influence (Bronk, 1939; Lorente de No', 1939; Lorente de No' and Laporte, 1950; Eccles, 1953). Some observations of Toman (1949) on the effect of tetanization on fiber threshold are therefore relevant. Toman noted a decrease in threshold of frog sciatic nerve lasting several minutes following a brief series of supramaximal repetitive shocks. Decrease in threshold of the afferent peripheral axons cannot account for PTP since usually all relevant fibers are stimulated before, during and after the tetanus. If, however, this reduction in threshold also occurred in the presynaptic terminals, a portion of those terminals in which conduction normally failed would be enabled to exert their excitatory action. In such a situation more mediator

would be released by a maximal afferent volley and a greater number of post-synaptic units would respond. Dilantin (Toman, 1955), as well as other anti-convulsant agents (Toman, 1949), is capable of preventing this decrease in threshold induced by tetanization. It is proposed that the action of Dilantin in reducing PTP is best explained by this mechanism.

This hypothesis is capable of being tested by appropriate experimental technics, and such experiments may shed light on the other hypotheses which have been tentatively rejected in the foregoing discussion. Information suggestive of a discrete effect of Dilantin on neuronal membranes which might be utilized to support the proposed mechanism is lacking; however, Woodbury (1955) has obtained evidence strongly suggesting that Dilantin increased the activity of the membrane sodium pump. On the basis of present information, a firm causal relationship between this action and the proposed mechanism cannot be drawn, but this observation is of value in channeling the course of future experiments.

Post-tetanic potentiation is most convincingly revealed by experimental technics which are rarely simulated physiologically: iterative impulses are delivered to the synapse following abrupt cessation of an intense, maximal, synchronous afferent barrage. This is not to say, however, that the phenomenon is a laboratory curiosity. The bulk of evidence indicates that PTP, including its subtle manifestations, is a normal consequence of synaptic activity and can be demonstrated after a very few 'tetanic' stimuli (Lloyd, 1949a; Eccles and Rall, 1951a, b; Eccles, 1953). Thus, PTP would appear to be a phenomenon similar to summated synaptic depression, though opposite in sense; the latter process would tend to restrict synaptic activity while

the former would act to extend and maintain it. Considered in this light, PTP may be significantly concerned in all functions of the nervous system characterized by repetitive activity, and the significance of its role would appear to be in direct relation to the intensity of the activity. Ward et al. (1955) have shown that individual neurons in epileptogenic foci (monkeys) may fire in prolonged bursts at frequencies as high as 500 impulses per second. Maximal seizure activity is terminated by depression of the relevant neurons (e.g., Toman and Taylor, 1952), and this depression necessarily outlasts and masks any potentiating process which may occur. The neurons concerned in seizure activity would indeed be unique if a continuous potentiation were not acting regeneratively to maintain and extend the activity once initiated. Should Dilantin be shown to effect PTP in cortical and subcortical synapses concerned with seizure activity, as it does synapses of the spinal cord and stellate ganglion, this action of the drug would appear clearly to be implicated as one component of its anti-convulsant effectiveness.

VI. SUMMARY

The effect of diphenylhydantoin (Dilantin) on various aspects of synaptic transmission in the spinal cord and stellate ganglion of cats has been investigated by electrophysiological techmics. Dilantin has a slight depressant effect upon transmission of isolated impulses in the spinal cord, and the effect upon activity in polysynaptic pathways in somewhat more pronounced. No effect of Dilantin on facilitation in either the spinal cord or ganglion has been observed; primary inhibition has not been thoroughly studied.

The principal effects of Dilantin on synaptic transmission were found to be qualitatively identical for the monosynaptic pathways of the spinal cord and ganglion. Dilantin deepens depression following transmission of a single impulse and hastens fatigue during repetitive stimulation. Post-tetanic potentiation is strikingly reduced by Dilantin. The doses employed in all studies were in the anticonvulsant, nontoxic range for cats.

In view of the anatomical and functional differences of the two synaptic systems studied and of the strikingly similar effects of Dilantin on transmission at these sites, it appears likely that the drug exerts similar effects upon synaptic transmission at sites in the central nervous system concerned with convulsive activity. The possible significance of the observed effects in relation to the anticonvulsant action of the drug is discussed.

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VIII. REFERENCES

Bernhard, C. G. Reflex patterns in interruncial systems. Assoc. Res. Nerv. & Ment. Dis., 1952, 30, 68-86.

Bradley, K., Easton, D. M. and Eccles, J. C. An investigation of primary or direct inhibition. J. Physiol., 1953, 122, 474-488.

Bradley, K. and Eccles, J. C. Analysis of the fast afferent impulses from thigh muscles. J. Physiol., 1953, 122, 462-473.

Bronk, D. W. Synaptic mechanisms in sympathetic ganglia. J. Neurophysiol., 1939, 2, 380-401.

Brooks, C. McC., Downman, C. B. B. and Eccles, J. C. After-potentials and excitability of spinal motoneurones following orthodromic activation. J. Neurophysiol., 1950, 13, 157-176.

Brooks, C. McC., and Eccles, J. C. An analysis of synaptic excitatory action. J. Neurophysiol., 1948, 11, 365-376.

Butler, T. C. Theories of general anesthesia. Pharmacol. Rev., 1950, 2, 121-160.

Castro, F. de. [Anatomical aspects of the ganglionic synaptic transmission in mammalians.] Arch. internat. physiol., 1951, 59, 479-525.

Creed, R. S., Denny-Brown, D., Eccles, J. C., Liddell, E. G. T. and Sherrington, C. S. Reflex activity of the spinal cord. Oxford University Press, London, 1932.

Downman, C. B. B., Eccles, J. C. and McIntyre, A. K. Functional changes in chromatolysed motoneurones. J. Comp. Neurol., 1953, 98, 9-36.

Eccles, J. C. The Neurophysiological basis of mind. The Clarendon Press, Oxford, 1953.

Eccles, J. C. and Rall, W. Effects induced in a monosynaptic reflex path by its activation. J. Neurophysiol., 1951, 14, 353-376.

Eccles, J. C. and Rall, W. Repetitive monosynaptic activation of motoneurones. Proc. Royal Soc., London, s. B., 1951, 138, 475-498.

Eccles, Rosamond M. Action potentials of isolated mammalian sympathetic ganglia. J. Physiol., 1952, 117, 181-195.

Eccles, Rosamond M. Responses of isolated curarized sympathetic ganglia. J. Physiol., 1952, 117, 196-217.

Esplin, D. W., Nicholes, K. R. K., Barrett, D., Hales, R. H., Petersen, L. J. and Washburn, J. B. Pharmacological studies on impulse transmission in the stellate ganglion. Unpublished observations, 1955.

Fatt, P. Biophysics of junctional transmission. Physiol. Rev., 1954, 34, 674-710.

Hagbarth, K.-E. Excitatory and inhibitory skin areas for flexor and extensor motoneurons. Acta physiol. scandinav., 1952, 94, Supp. 26, 1-38.

Harreveld, A. van and Feigen, G. A. Effect of some drugs on the polarization state of spinal cord elements. Am. J. Physiol., 1950, 160, 451-461.

Harreveld, , A. van, Foster, R. J. and Fasman, G. D. Effect of diphenylhydantoin (Dilantin) on ether and pentobarbitol (Nembutal) narcosis. Am. J. Physiol., 1951, 166, 718-722.

Harris, A. S. and Kokernot, R. H. Effects of diphenylhydantoin sodium (Dilantin sodium) and phenobarbitol sodium upon ectopic ventricular tachycardia in acute myocardial infarction. Am. J. Physiol., 1950, 163, 505-516.

Hunt, C. C. Relation of function to diameter in afferent fibers of muscle nerves. J. Gen. Physiol., 1954, 38, 117-131.

Jefferson, A. and Benson, A. Some effects of post-tetanic potentiation of monosynaptic response of spinal cord of cat. J. Neurophysiol., 1953, 16, 381-396.

Jefferson, A. A. and Schlapp, W. Some effects of repetitive stimulation of afferents on reflex conduction. In: The spinal cord (J. L. Malcolm and J. A. B. Gray, editors). Little, Brown and Co., Boston, 1953.

Job, C., and Lundberg, A. On the significance of post- and pre-synaptic events for facilitation and inhibition in the sympathetic ganglion of the cat. Acta physiol. scandinav., 1953, 28, 14-28.

Kaada, B. R. Site of action of myanesin (mephenesin, tolserol) in the central nervous system. J. Neurophysiol., 1950, 13, 89-104.

King, Ellen Eva, and Unna, K. R. The action of mephenesin and other interneuron depressants on the brain stem. J. Pharmacol. & Exper. Therap., 1954, 111, 293-301.

Koizumi, Kiyomi, Malcolm, J. L., and Brooks, C. McC. Effect of temperature on facilitation and inhibition of reflex activity. Am. J. Physiol., 1954, 179, 507-512.

Korey, S. R. Effect of Dilantin and Mesantoin on the giant axon of the squid. Proc. Soc. Exper. Biol. & Med., 1951, 76, 297-299.

Laporte, Y. Disynaptic reflex linkage between certain muscles of the hindlimb. In: The spinal cord (J. L. Malcolm and J. A. B. Gray, editors). Little, Brown, and Co., Boston, 1953.

Larrabee, M. G. and Bronk, D. W. Prolonged facilitation of synaptic excitation in sympathetic ganglia. J. Neurophysiol., 1947, 10, 139-154.

Latimer, C. N. Depression of transmission across single synapses by mephenesin. Fed. Proc., 1955, 14, 91.

Lorente de No', R. Transmission of impulses through cranial motor nuclei. J. Neurophysiol., 1939, 2, 402-464.

Lorente de No', R. and Laporte, Y. Synaptic transmission in a sympathetic ganglion. J. Cell. & Comp. Physiol., 1950, 35, Supp. 2, 1-192.

Lloyd, D. P. C. Reflex action in relation to pattern and peripheral source of afferent stimulation. J. Neurophysiol., 1943, 6, 111-119.

Lloyd, D. P. C. Neuron patterns controlling transmission of ipsilateral hind limb reflexes in the cat. J. Neurophysiol., 1943, 6, 293-315.

Lloyd, D. P. C. Conduction and synaptic transmission of the reflex response to stretch in spinal cats. J. Neurophysiol., 1943, 6, 317-326.

Lloyd, D. P. C. Functional organization of the spinal cord. Physiol. Rev., 1944, 24, 1-17.

Lloyd, D. P. C. Facilitation and inhibition of spinal motoneurons. J. Physiol., 1946, 9, 421-438.

Lloyd, D. P. C. Integrative pattern of excitation in two-neuron reflex arcs. J. Neurophysiol., 1946, 9, 439-444.

Lloyd, D. P. C. Post-tetanic potentiation of response in monosynaptic reflex pathways of the spinal cord. J. Gen. Physiol., 1949, 33, 147-170.

Lloyd, D. P. C. In: A textbook of physiology (Fulton, J. C., editor). W. B. Saunders Co., Philadelphia, 1949, Chapters III & IV.

Lloyd, D. P. C. Electrical signs of impulse conduction in spinal motoneurons. J. Gen. Physiol., 1951, 35, 289-319.

Pedersen, E. Studies on the central pathway of the flexion reflex in man and animal. Acta psychiat. et neurol. scandinav., 1954, Supp. 88, 1-81.

Perry, W. L. M. Acetylcholine release in the cat's superior cervical ganglion. J. Physiol., 1953, 119, 439-454.

Putnam, T. J. and Merritt, H. H. Chemistry of anticonvulsant drugs. Arch. Neurol. & Psychiat., 1941, 45, 505-516.

Reighard, J. and Jennings, H. S. Anatomy of the cat. Henry Holt & Co., New York, 1949.

Renshaw, B. Activity in the simplest spinal reflex pathways. J. Neurophysiol., 1940, 3, 373-387.

Renshaw, B. Reflex discharges in branches of the crural nerve. J. Neurophysiol., 1942, 5, 487-498.

Rosenblueth, A. The transmission of nerve impulses at neuroeffector junctions and peripheral synapses. John Wiley & Sons, Inc., New York, 1950.

Scherf, D. Changes in the electrocardiogram after intravenous administration of phenytoin sodium (Dilantin) in the acute experiment. Bull. N. Y. Med. College, Flower & Fifth Ave. Hosp., 1943, 6, 82-89.

Swinyard, E. A., Brown, W. C. and Goodman, L. S. Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. & Exper. Therap., 1953, 106, 319-330.

Toman, J. E. P. The neuropharmacology of antiepileptics. EEG Clin. Neurophysiol., 1949, 1, 33-44.

Toman, J. E. P. Neuropharmacology of peripheral nerve. Pharmacol. Rev., 1952, 4, 168-218.

Toman, J. E. P. Personal communication, 1955.

Toman, J. E. P., Swinyard, E. A. and Goodman, L. S. Properties of maximal seizures, and their alteration by anticonvulsant drugs and other agents. J. Neurophysiol., 1946, 9, 231-240.

Toman, J. E. P. and Taylor, J. D. Mechanism of action and metabolism of anticonvulsants. Epilepsia, Third Series, 1952, 1, 31-48.

Ward, A. A., Jr., Thomas, L. B. and Schmidt, R. P. Activity of single cortical elements in epileptic foci of monkey. Fed. Proc., 1955, 14, 158.

Woodbury, D. M. Effect of diphenylhydantoin on electrolytes and radiosodium turnover in brain and other tissues of normal, hyponatremic and postictal rats. J. Pharmacol. & Exper. Therap., 1955, in press.

Wright, E. B. Effect of mephenesin and other 'depressants' on spinal cord transmission in frog and cat. Am. J. Physiol., 1954, 179, 390-401.

Fig. 1. Schema of some of the muscles and neuron pathways in the ankle-joint myotatic unit of the cat.

Facilitatory afferent collaterals are shown by the fine solid lines and inhibitory collaterals by the fine broken lines. M. H. G., medical head of gastrocnemius; L. H. G., lateral head of gastrocnemius; T. A., tibialis anterior., m., motoneuron; m. s., muscle spindle; m. e., motor nerve endings in muscle. S, stimulating electrodes; R, recording electrodes. Not all electrodes were placed in a given experiment. When dorsal root stimulation was employed, the root was cut proximal to the ganglion.

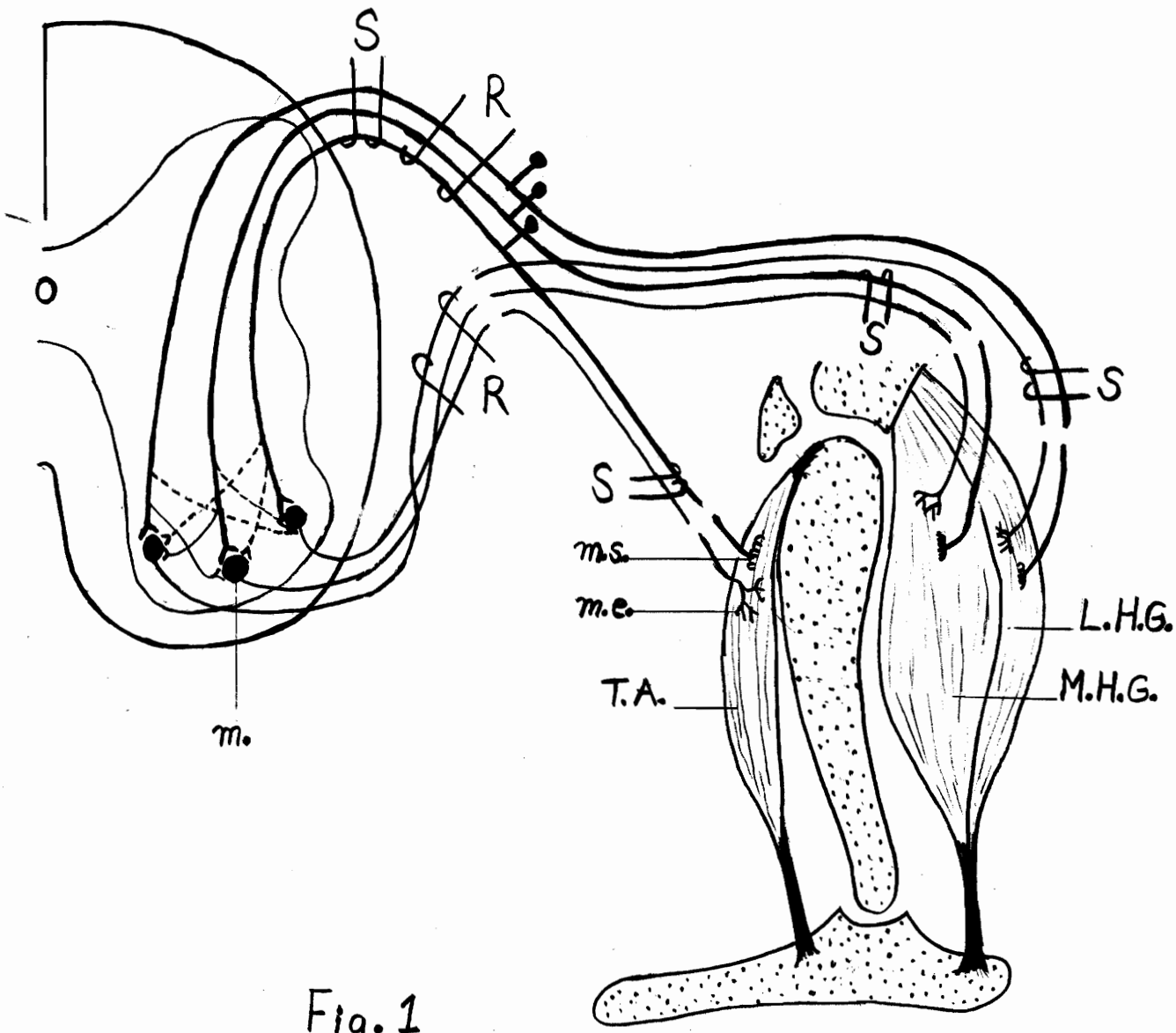

Fig. 1

Fig. 2. Effects of dilantin on transmission of isolated impulses in the spinal cord.

A. DR-VR preparation (experiment S-12). 1, control; 2, 3, and 4, after 10, 20 and 30 mgm./kgm. Dilantin, respectively. Gain and sweep speed constant throughout series.

B. DR-VR preparation (experiment S-11). 1, control; 2, 3 and 4, after 10, 20 and 30 mgm./kgm. Dilantin, respectively. Gain is constant but sweep speed varied.

C. Nerve to medial head of gastrocnemius stimulated. 1 and 2, control; 3 and 4, after 30 mgm./kgm. Dilantin. Gain constant throughout; sweep speed was changed after Dilantin. (Experiment S-10)

D. Sural nerve stimulated. 1 and 2, control; 3 and 4, after 10 mgm./kgm. Dilantin. Gain in 3 and 4 is 2.5 times gain of control. Sweep speed was changed after Dilantin. Response 2 traced with ink to improve contrast. (Experiment S-7)

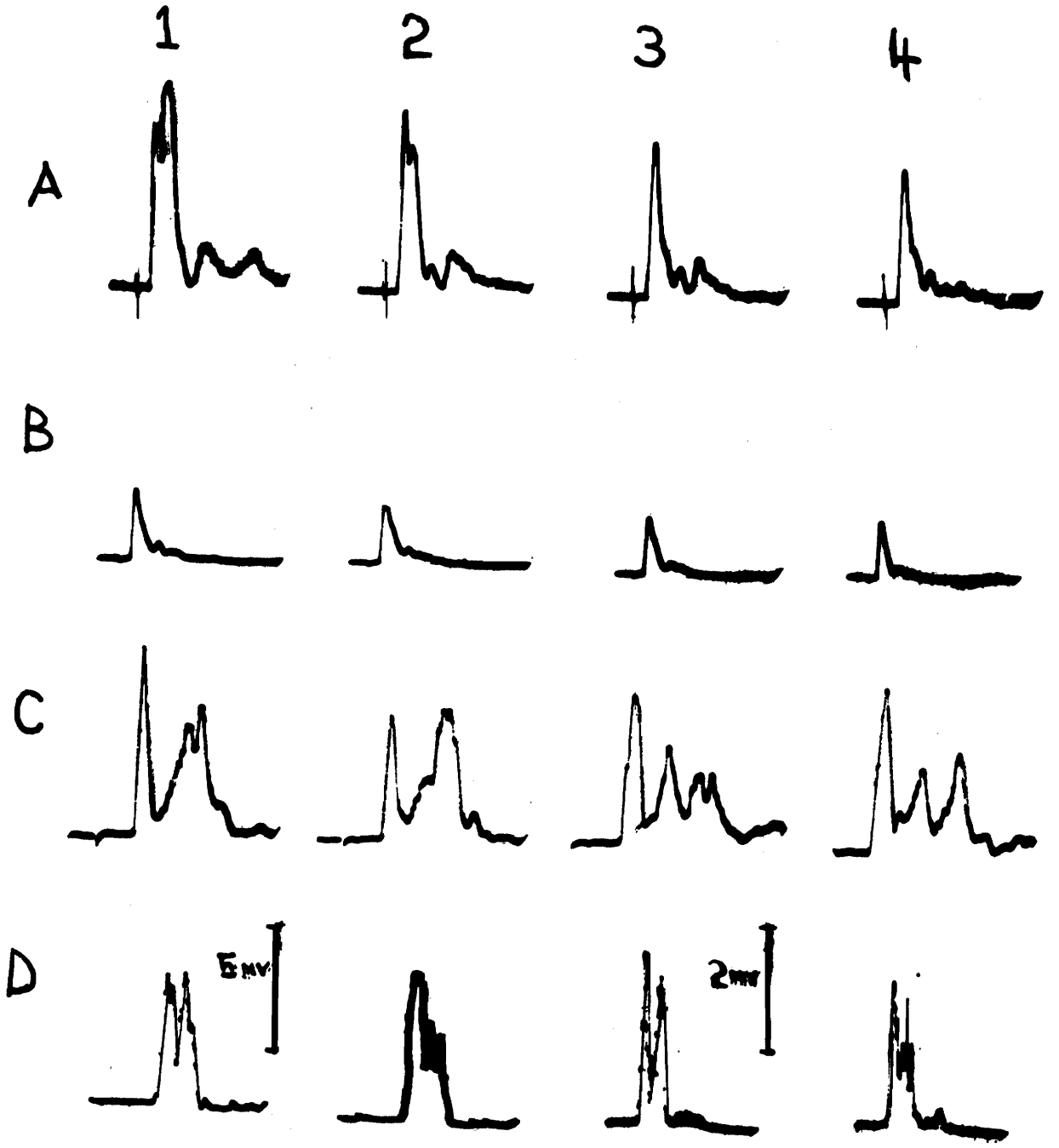

Fig.2

Fig. 3. Effect of Dilantin on monosynaptic facilitation in the spinal cord.

Ordinate, per cent of respective control monosynaptic spike height; abscissa, interval in milliseconds between conditioning and test s timuli. Conditioning stimulus, subliminal for 2N response, applied to nerve to medial head of gastrocnemius; test stimulus, supramaximal for 2N response, applied to nerve to lateral head of gastrocnemius. Solid line, control; broken line, 30 minutes after 30 mgm./kgm. Dilantin. Each point is average of about 4 responses. Dilantin injected at the rate of 6 mgm./kgm./ minute. (Experiment S-1)

Fig. 4. Effect of Dilantin on monosynaptic inhibition.

Ordinate, per cent of respective control monosynaptic spike height; abscissa, interval in milliseconds between contitioning and test stimuli, corrected for difference in conduction time. Subliminal conditioning stimulus applied to tibialis anterior nerve; supramaximal test stimulus applied to nerve to medial head of gastrocnemius. Solid line, control; broken line, 2 hours after 10 mgm./kgm. Dilantin. Each point is average of about 4 response. (Experiment S-7)

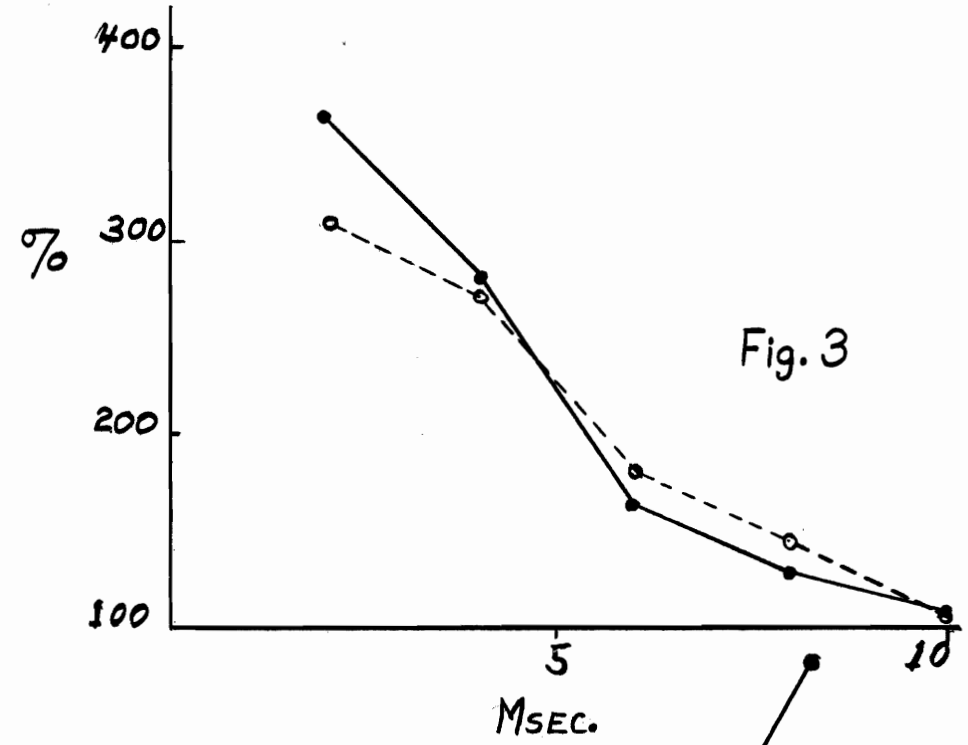

Fig. 3

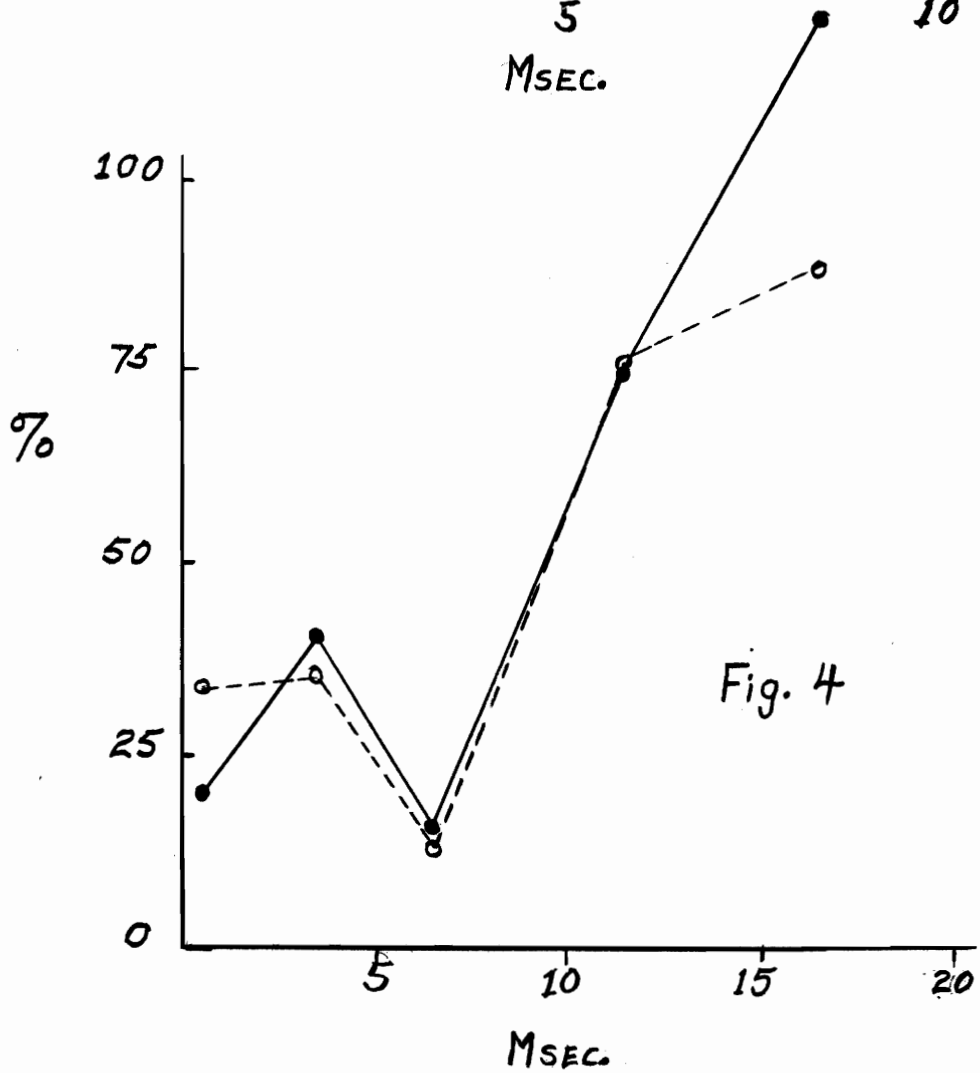

Fig. 4

Fig. 5. Effect of Dilantin on synaptic depression.

DR-VR preparation (S-11). Ordinate, per cent of respective control monosynaptic spike height; abscissa, interval in milliseconds between two supramaximal dorsal root stimuli.

Fig. 6. Effect of Dilantin on response to repetitive stimulation.

DR-VR preparation (S-11). Ordinate, per cent of respective control monosynaptic spike height; abscissa, number of response in the train.

A. Dorsal root stimulated supramaximally at frequency of 15/minute.

B. Dorsal root stimulated supramaximally at frequency of 65/minute.

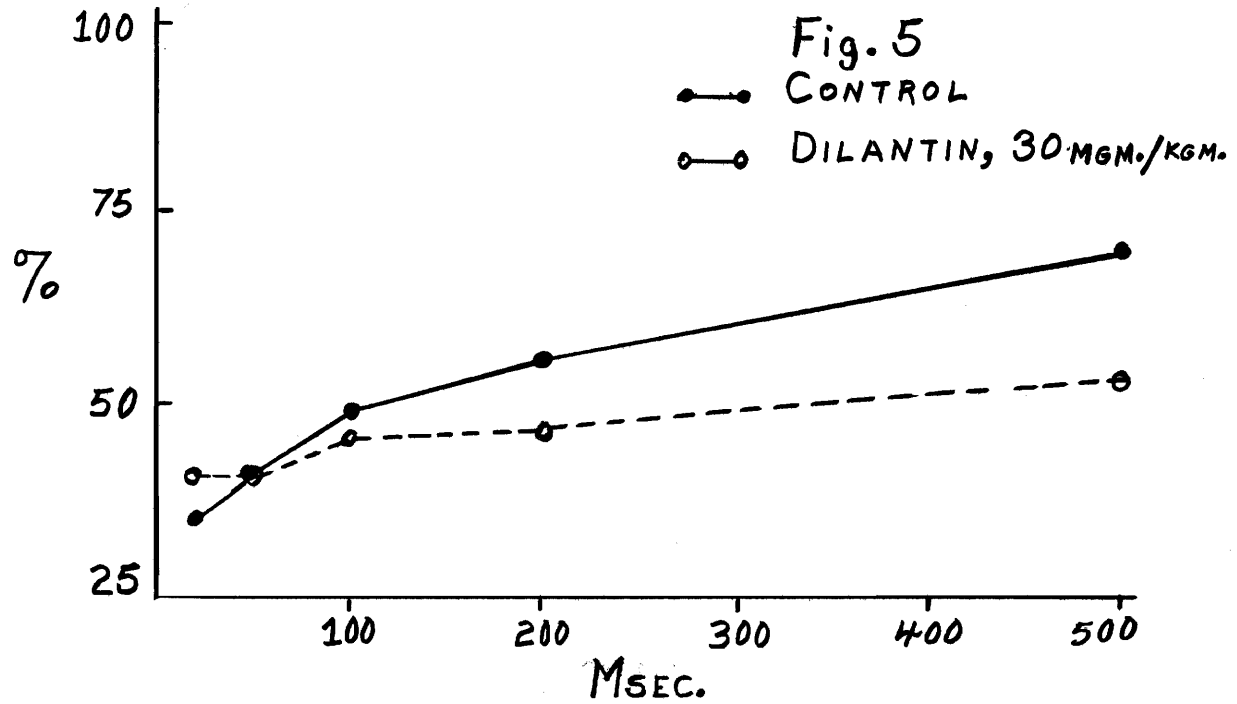
100
75
50
25
%
100
200
300
400
500
MSEC.
CONTROL
DILANTIN, 30 MGM./KGM.

Fig. 5

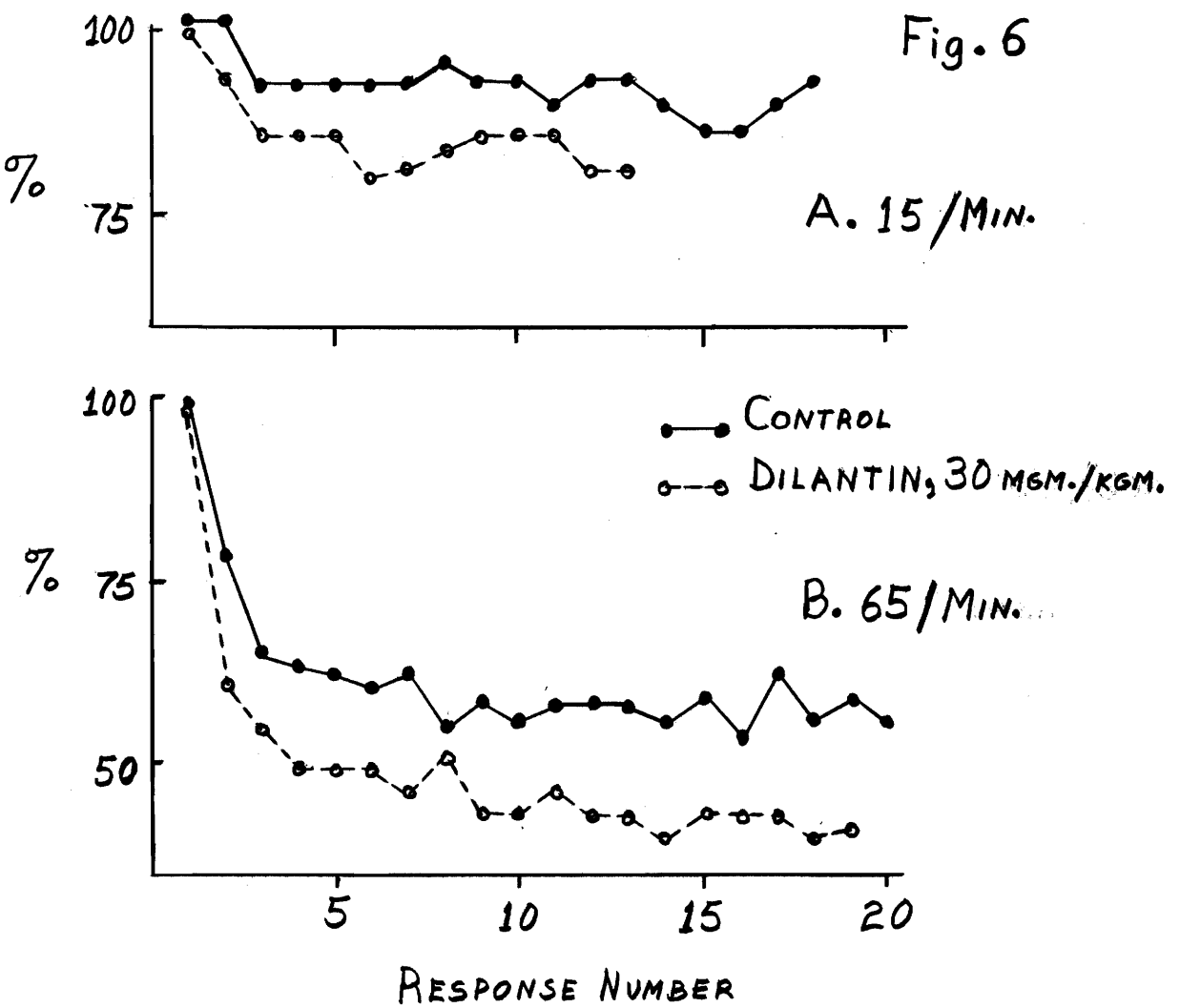
100
75
%
A. 15/MIN.
100
75
50
%
B. 65/MIN.
CONTROL
DILANTIN, 30 MGM./KGM.
5
10
15
20
RESPONSE NUMBER

Fig. 6

Fig. 7. Stability of spinal cord PTP with time.

DR-VR preparation (S-11). Ordinate, monosynaptic spike in millivolts; abscissa, time in seconds after dorsal root tetanus of 500/sec. for 15 seconds. a, control PTP at 4:36 P.M.; b, control PTP at 8:05 P.M. In this and later PTP curves, respective pretetanic control spike heights are indicated by lines on ordinate.

Fig. 8. Effect of Dilantin on spinal cord PTP induced by muscle-nerve stimulation.

Tetanic and test stimuli applied to nerve to medial head of gastrocnemius. Tetanus, 300/sec. for 15 seconds. a, control; b, after 10 mgm./kgm. Dilantin; c, after 20 mgm./kgm. Dilantin. (Experiment S-7)

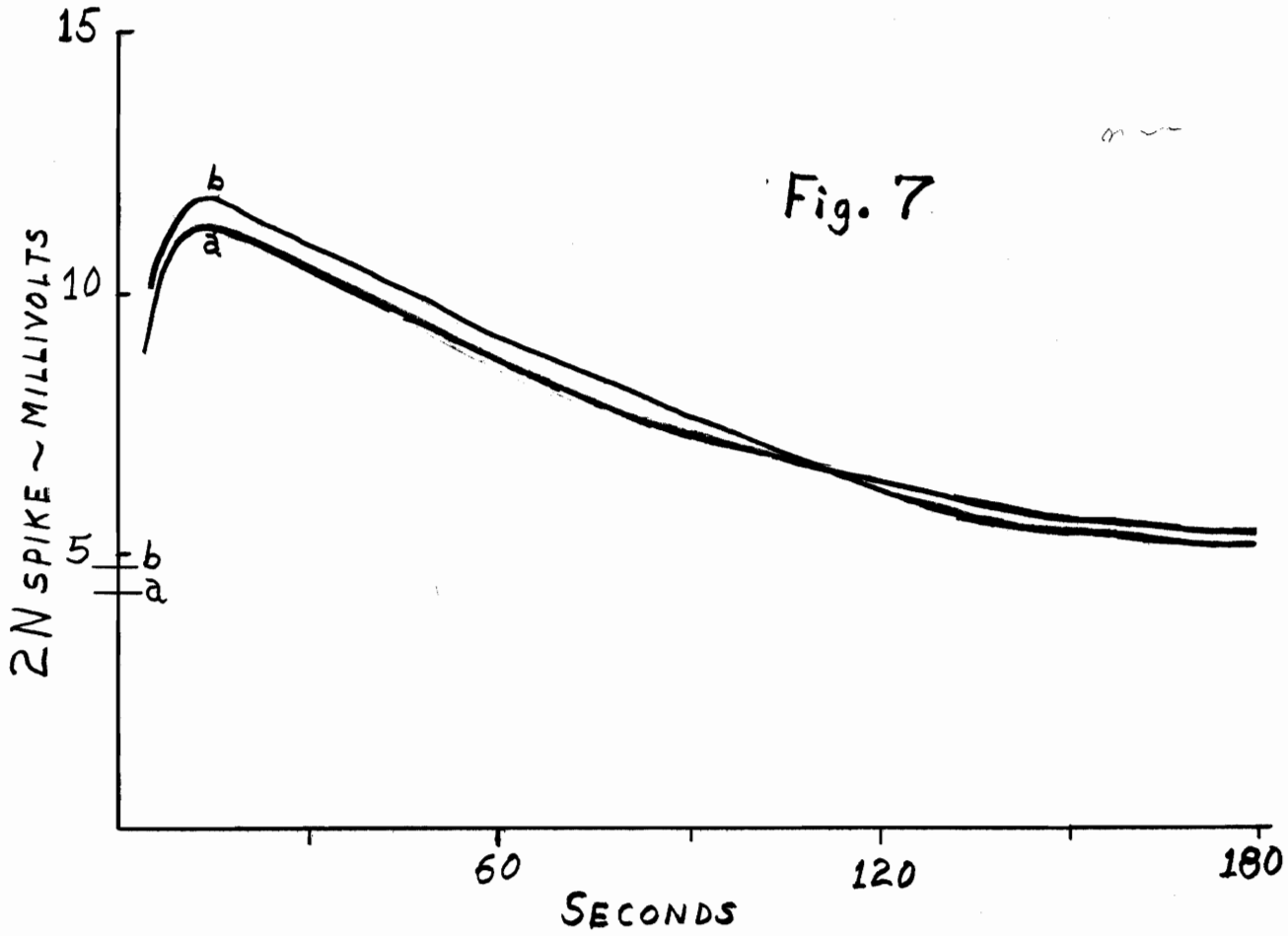
15
10
5
b
a
b
a
2N SPIKE ~ MILLIVOLTS
60
120
180
SECONDS

Fig. 7

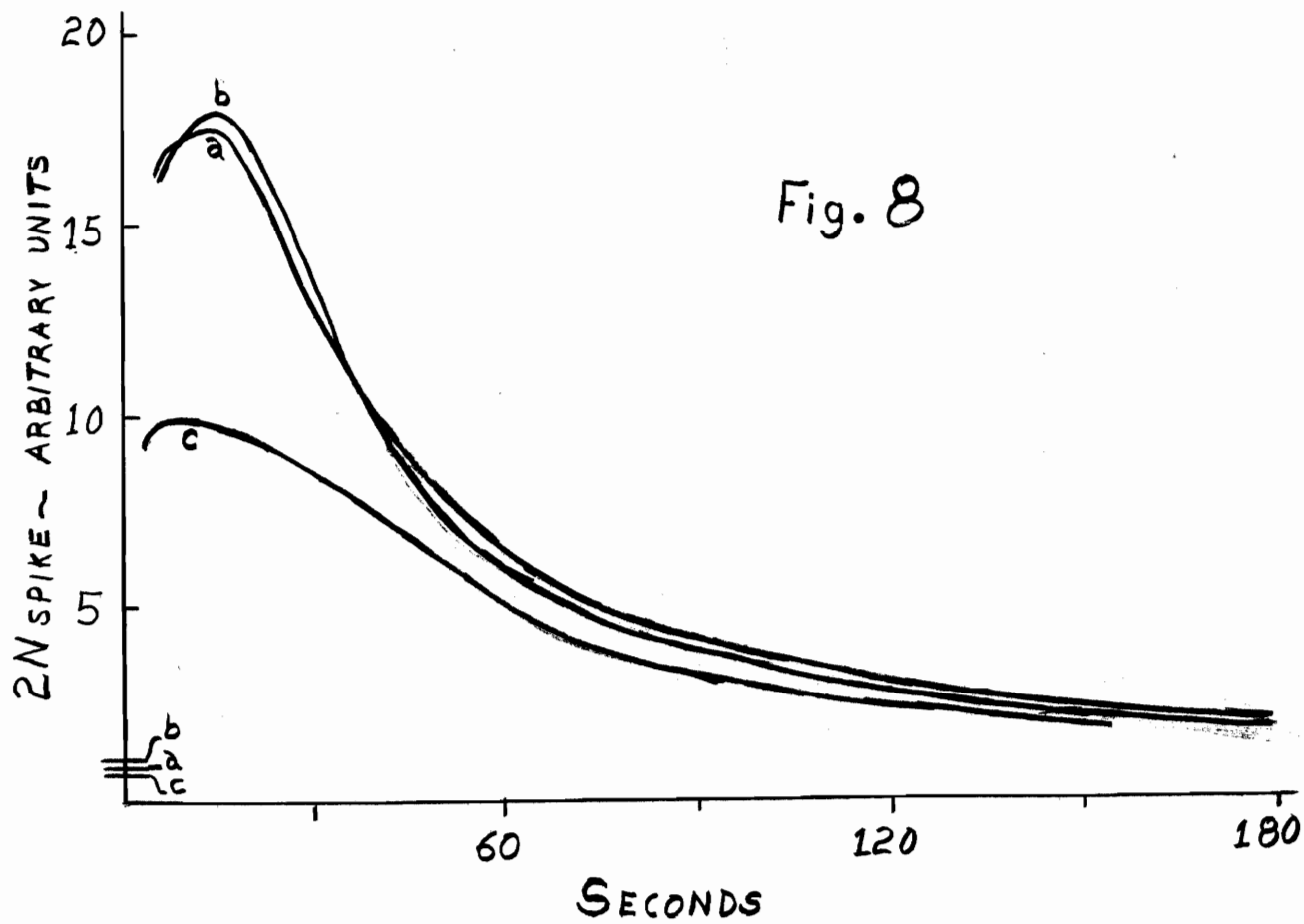
20
15
10
5
b
a
c
b
a
c
2N SPIKE ~ ARBITRARY UNITS
60
120
180
SECONDS

Fig. 8

Fig. 9. Effect of various doses of Dilantin on spinal cord PTP.

DR-VR preparation (S-11). a, control PTP, same curve as figure 7 curve b (selected responses shown in figure 13 B); b, c, d, and e, after 10, 20, 30 and 40 mgm./kgm. Dilantin, respectively. Individual points included for curve c, to show dispersion typically observed. Selected responses for curve d shown in figure 13 C.

Fig. 10. Variations in spinal cord PTP with time after 30 mgm./kgm. Dilantin.

DR-VR preparation (S-11). a, PTP determined 5 minutes after bringing total dose to 30 mgm./kgm.; b and c, 1 hour and 13 hours, respectively, after this dose of Dilantin. Curve a is the same as curve d in figure 9. Note changes in level of excitability indicated by lines on the ordinate. The curves cross because, as PTP wanes, each curve tends toward its own baseline.

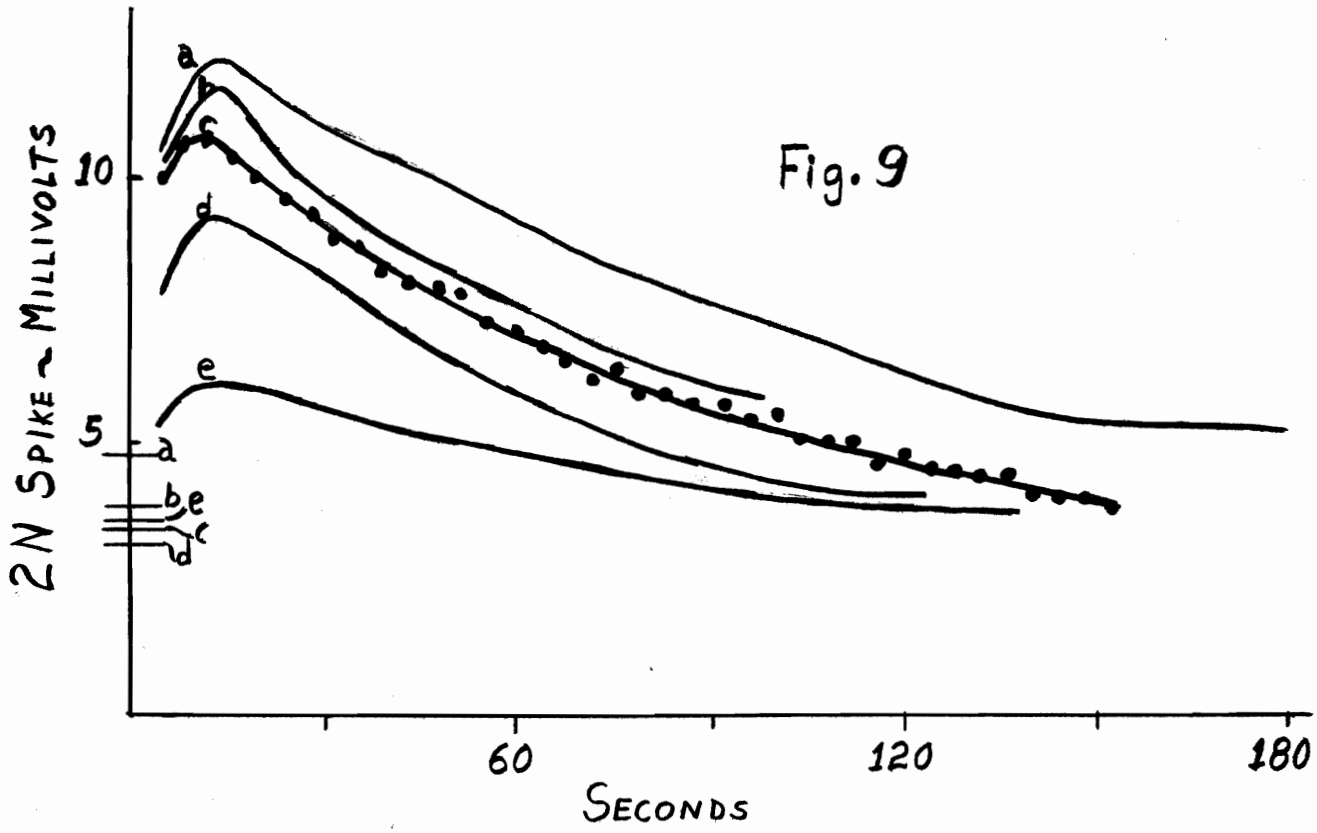
2N Spike ~ Millivolts
10
5
a
b
c
d
e
60
120
180
Seconds

Fig. 9

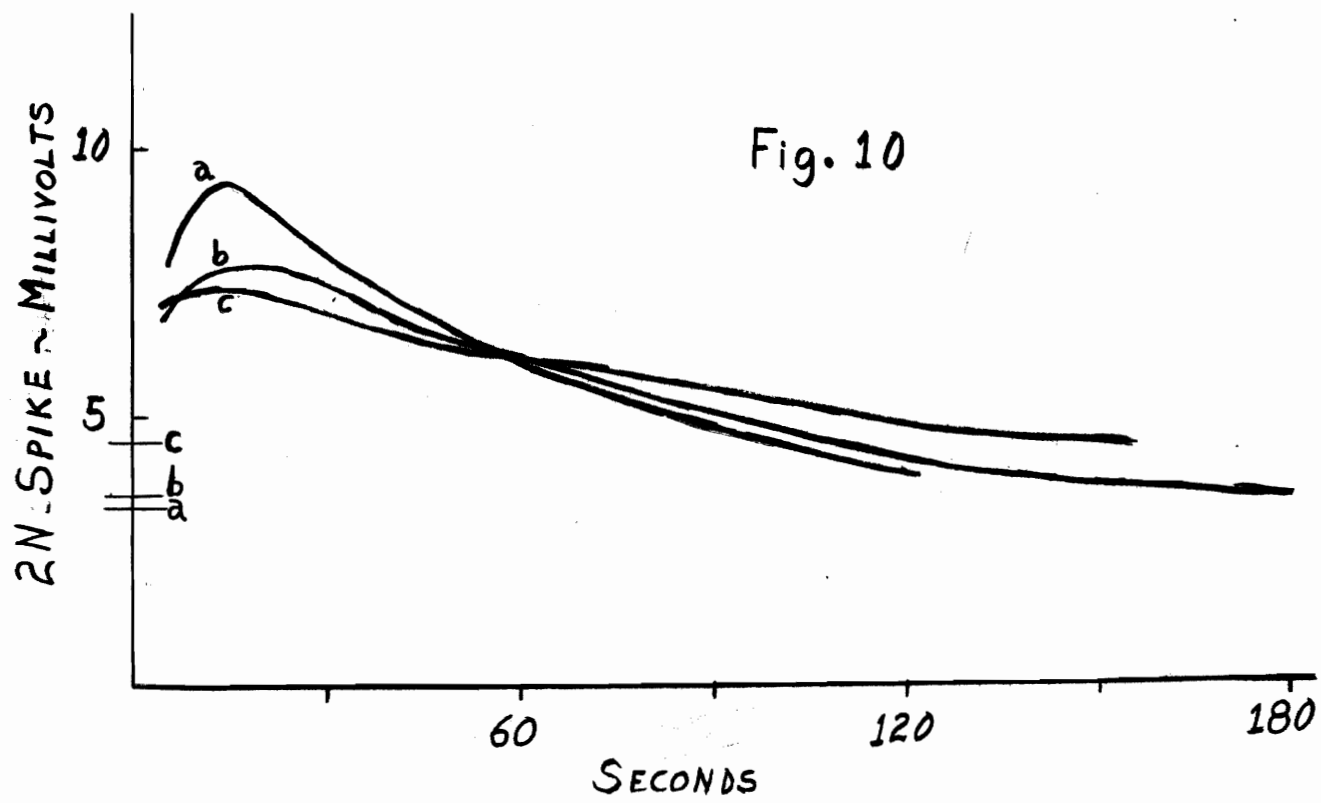
2N Spike ~ Millivolts
10
5
a
b
c
60
120
180
Seconds

Fig. 10

Fig. 11. Effect of Dilantin on spinal cord PTP induced by tetanic stimulation at various frequencies.

Nerve to medial head of gastrocnemius stimulated (experiment S-10). Tetanic stimulation was of 15 seconds duration at frequencies indicated on each curve. Refer to figure 2 C for representative control responses before and after administration of Dilantin. In each series, curves were determined in order of increasing frequency with 5-8 minutes between tetanizations. Responses monitored visually between determinations and several control responses were photographed at the beginning and at the end of each series. The averages of these control responses are indicated by the horizontal lines. Note the increase in control 2N spike height which occurred during determination of the Dilantin series. Thus, several of the curves in this series (higher frequencies) tend toward a higher baseline than curves for corresponding frequencies in the control series.

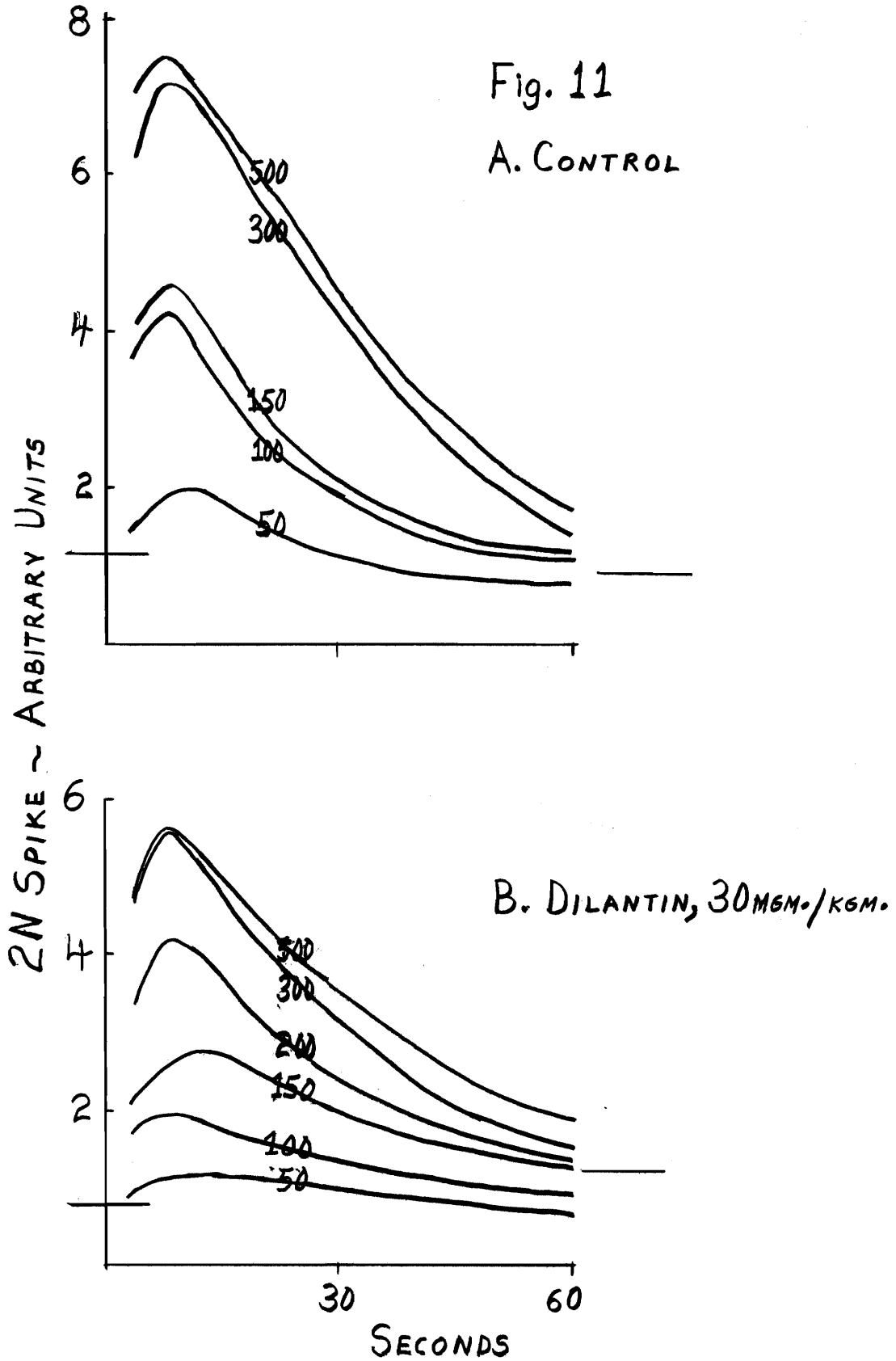
Fig. 11
A. Control
B. Dilantin, 30 mgm./kgm.
2N Spike ~ Arbitrary Units
8
6
4
2
500
300
150
100
50
6
4
2
500
300
200
150
100
50
30
60
Seconds

Fig. 12. Effect of tetanization on synaptic threshold in spinal cord.

DR-VR preparation (S-12). Curves in upper portion of graph are plots of ventral root 2N spike height against dorsal root spike height, both expressed as per cent of maximum. Dorsal root stimuli varied from below threshold to stimuli clearly supramaximal for the relevant afferent fibers. Open circles determined at resting level of excitability; each point is average of two determinations. Filled circles determined during the period from 8 to 40 seconds after a dorsal root tetanus of 500 stimuli/second for 15 seconds. Each point is one determination; a four-second interval was allowed between stimuli. Representative responses are shown in figure 13 D.

In the lower portion of the graph are shown two curves obtained by approximate graphical differentiation of the experimentally determined curves. These curves show the distributions of thresholds, in terms of number of afferent fibers activated, for the two-neuron pathway at rest and during PTP. Areas under the two curves are proportional to the respective maximum discharge zones under the two conditions.

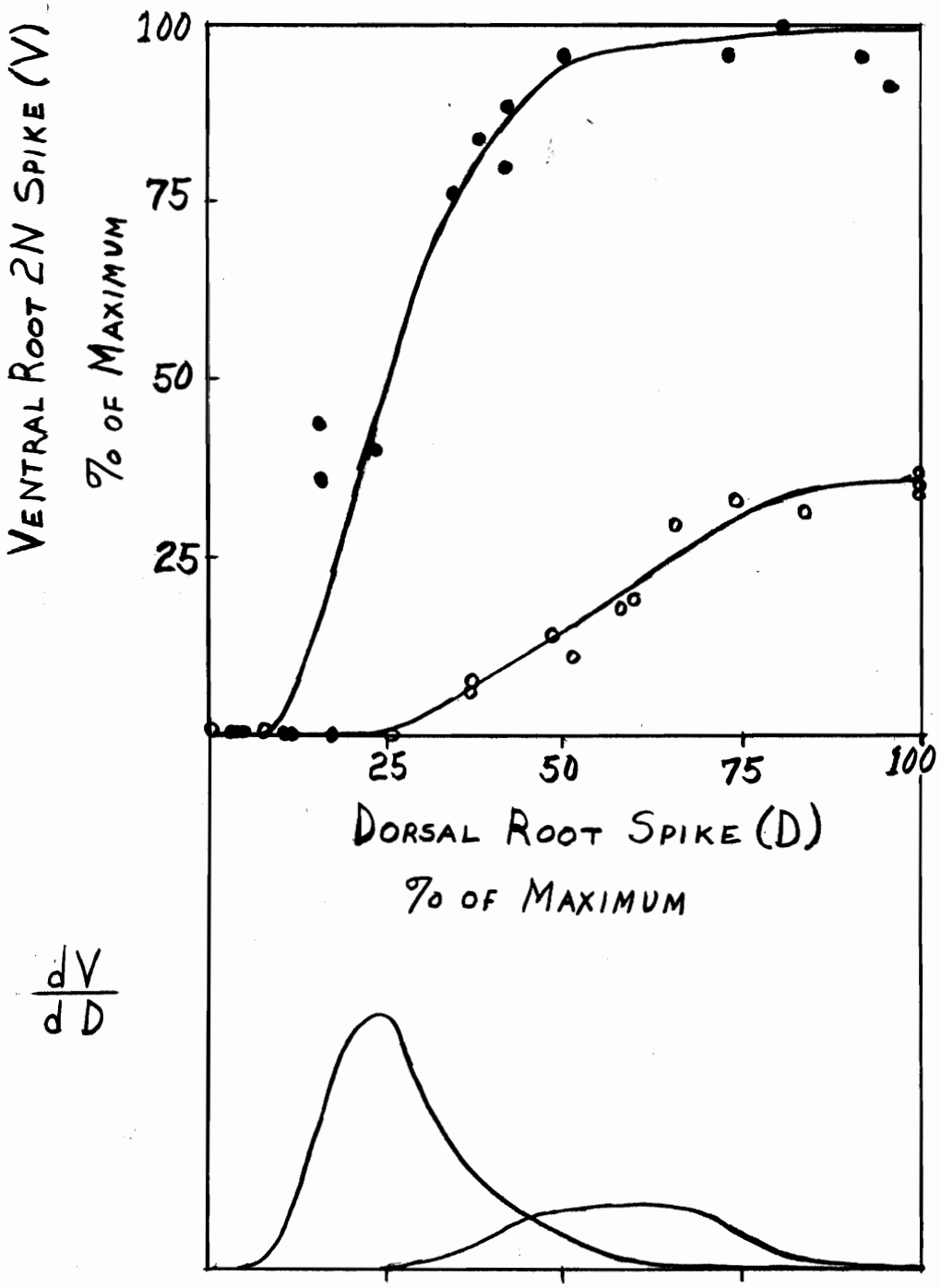
VENTRAL ROOT 2N SPIKE (V)
% OF MAXIMUM
100
75
50
25
25
50
75
100
DORSAL ROOT SPIKE (D)
% OF MAXIMUM
dV/dD

Fig. 12

Fig. 13. Spinal cord responses, illustrating selected phenomena.

A. Primary inhibition (figure 4, control series). 1, response to conditioning stimulus; 2, response to test stimulus; 3 and 4, inhibited responses at intervals of 3.5 and 10.5 msec., respectively. Gain and sweep speed constant. 500 cps. time signal.

B. Control PTP (figure 7 curve b, and figure 9 curve a). 1, pretetanic control response; 2, 3 and 4, potentiated responses at 12, 60 and 120 seconds after tetanus, respectively. Gain and sweep spread constant.

C. PTP after 30 mgm./kgm. Dilantin (figure 9 curve d, and figure 10 curve a). 1, pretetanic control response; 2, 3 and 4, potentiated responses at 12, 60 and 120 seconds after tetanus, respectively. Gain and sweep speed constant. Gain same as in B.

D. Effect of PTP on synaptic threshold (figure 12). Upper trace, dorsal root; lower trace, ventral root. 1 and 2, at rest; 3 and 4, during PTP. Sweep speed of both traces constant. Gain of upper trace constant; gain of lower trace in 1 and 2 is 2.5 times that in 3 and 4.

E. PTP in polysynaptic pathways. Sural nerve stimulated (experiment S-8). 1, pretetanic control response; 2, 3 and 4, potentiated responses at 12, 24 and 48 seconds after tetanus, respectively. Tetanus was 75 stimuli/second for 5 seconds. Gain and sweep speed constant.

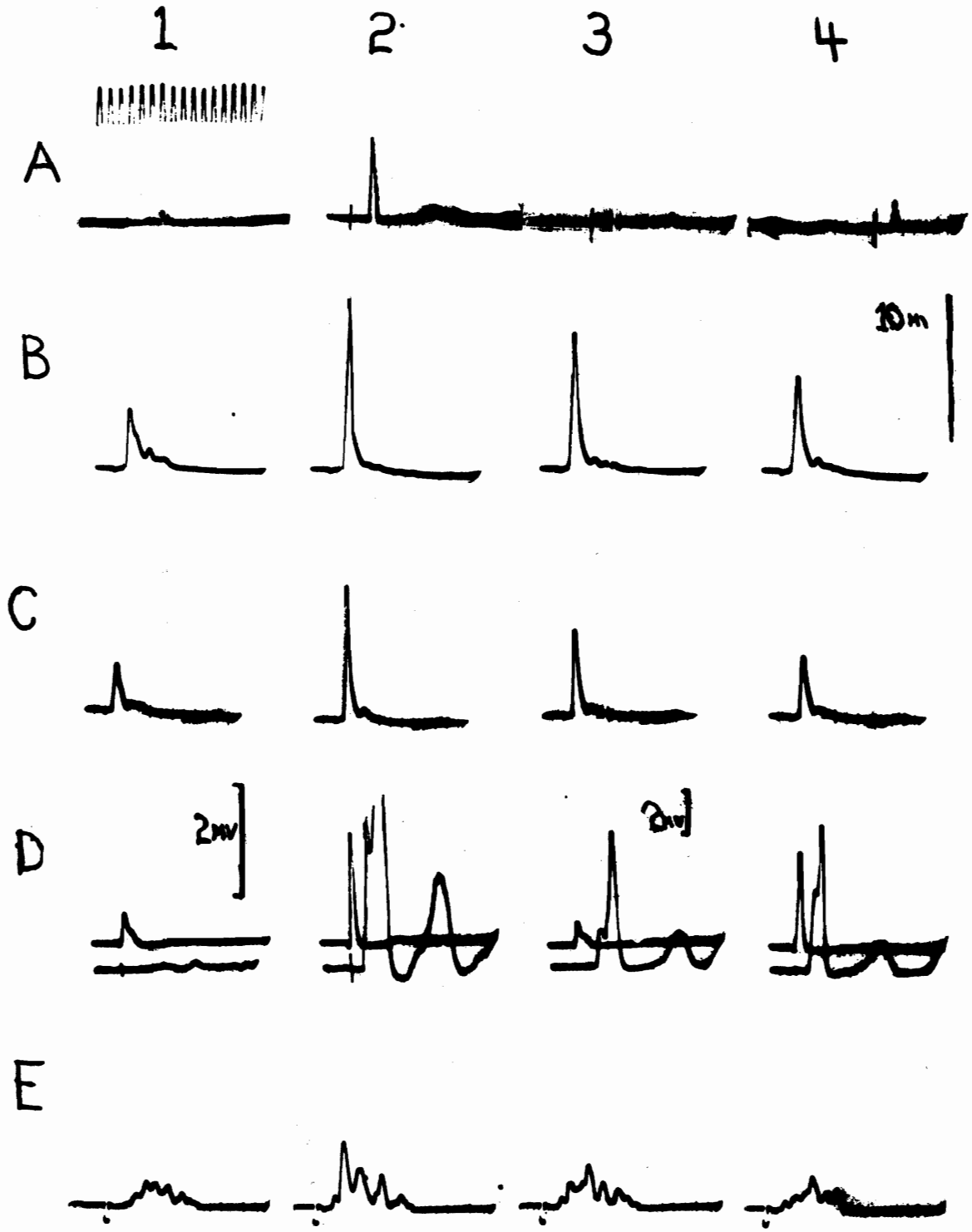

Fig. 13

Fig. 14. Schema of the stellate ganglion of the cat.

I-IV, thoracic rami; S. T., sympathetic trunk; I. C. N., inferior cardiac nerve; S, stimulating electrodes; R, recording electrodes.

Fig. 15. Representative postganglionic response.

A. Effect of Dilantin on transmission of isolated impulses. 1, control; 2, 3, and 4, after 10, 20 and 30 mgm./kgm. Dilantin, respectively. Time signal, 200 cps. Gain and sweep speed constant. (Experiment G-20)

B. PTP, control series. 1, pre tetanic control; 2, 3, and 4, potentiated responses 8, 16 and 60 seconds, respectively, after a tetanus of 15 stimuli/second for 5 seconds. Gain and sweep speed constant. (Experiment G-17)

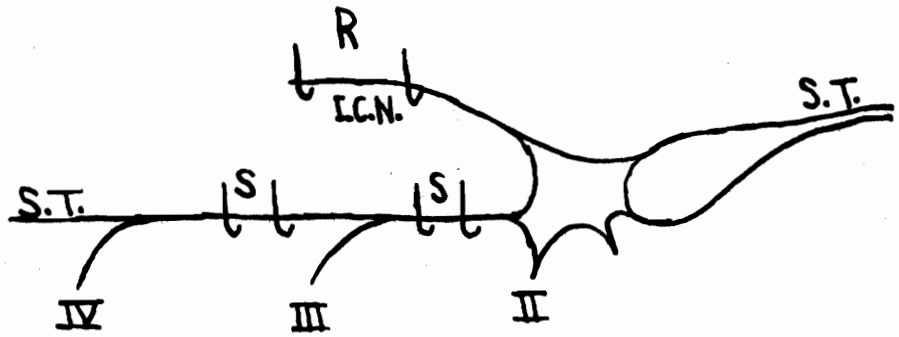

Fig.14

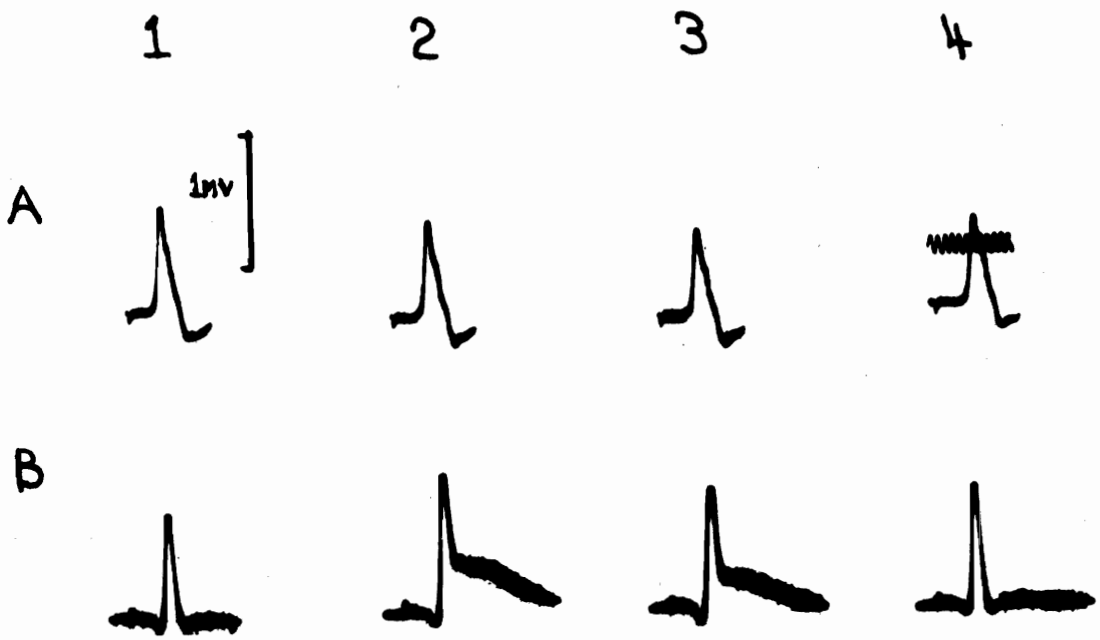

Fig.15

Fig. 16. Effect of Dilantin on synaptic depression following transmission of a single impulse in the stellate ganglion.

Ordinates, height of the second response expressed as per cent of the first; abscissa, time in milliseconds between stimuli. Each point is the average of 4 responses. From upper graph downwards, experiments G-17, G-18 and G-20.

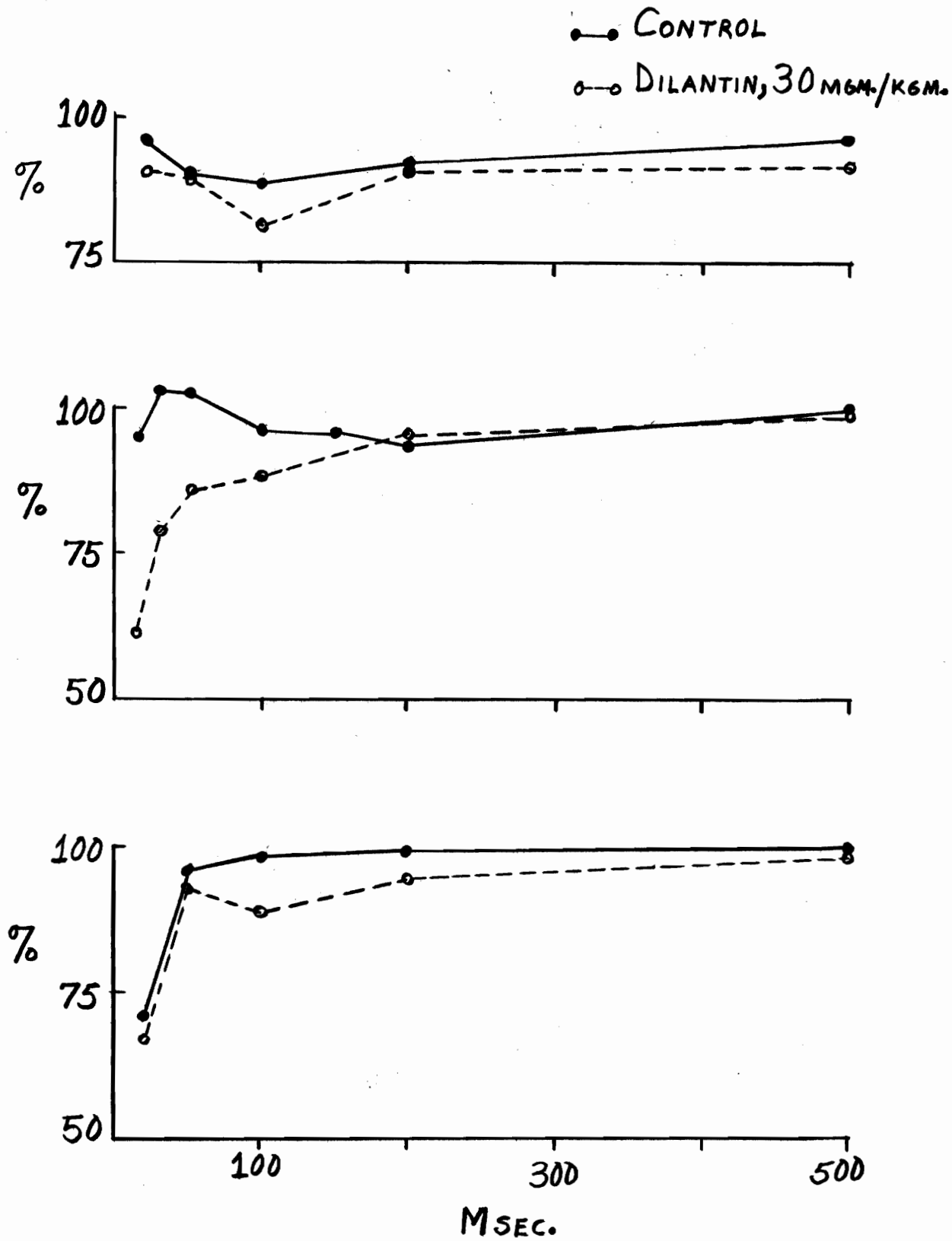
CONTROL
DILANTIN, 30 MGM./KGM.
100
%
75
100
%
75
50
100
%
75
50
100
300
500
MSEC.

Fig. 16

Fig. 17. Effect of Dilantin on postganglionic response to repetitive stimulation.

Ordinates, spike height in arbitrary units. The units are the same for the two curves of each graph. No significant change in height of control response occurred over the whole experiment. Recording artifact of moving film photography makes the units of the graph at 30/sec. different from the other graphs. Note also in the 30/sec. series that after Dilantin, the first few responses were missed. In this experiment Dilantin was injected at the rate of 1.4 mgm./kgm./minute. (Experiment G-6)

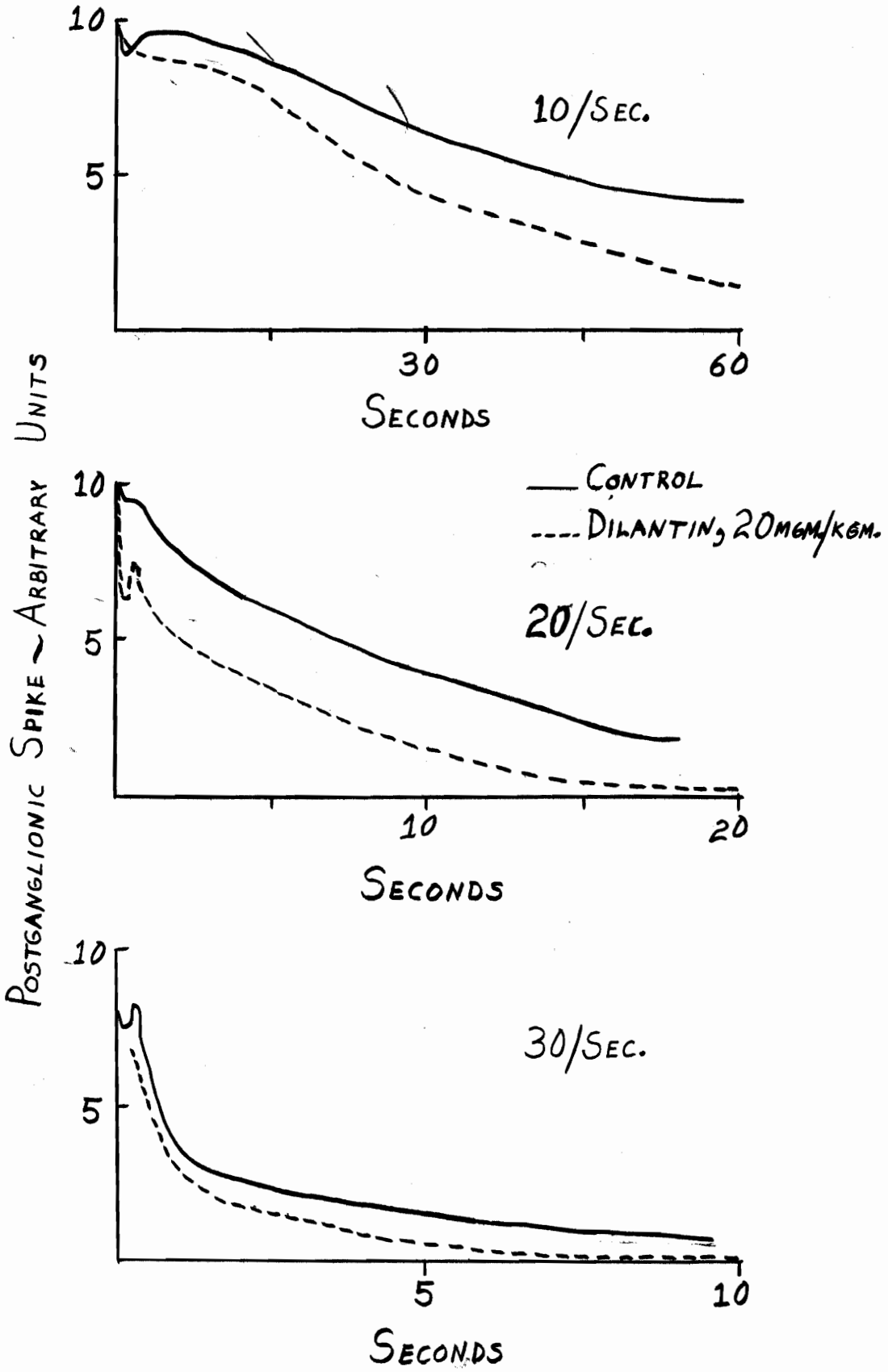
10
5
10/SEC.
30
60
SECONDS
POSTGANGLIONIC SPIKE ~ ARBITRARY UNITS
10
5
CONTROL
DILANTIN, 20MGM/KGM.
20/SEC.
10
20
SECONDS
10
5
30/SEC.
5
10
SECONDS

Fig. 17

Fig. 18. Effect of Dilanton on ganglionic PTP.

Tetanus 75/sec. for 5 sec. Experiment G-18; stimulating electrodes between rami III and IV. a, control (same as curve b, figure 18); b, c, and d, after 10, 20, and 30 mgm./kgm. Dilantin, respectively. Portions of records from which curves a and d were obtained are shown in figure 20.

Fig. 19. Effect of Dilantin on ganglionic PTP.

Tetanus 30/sec. for 10 sec. Experiment G-20; stimulating electrodes between rami III and IV. a, control; b, after 30 mgm./kgm. Dilantin.

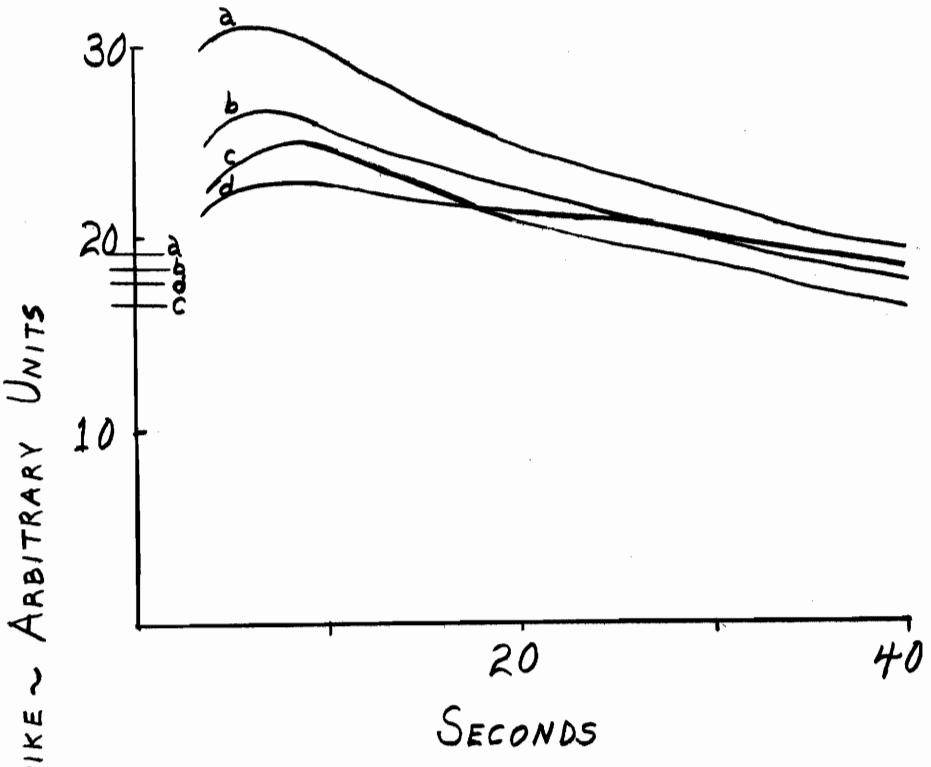
30
20
10
a
b
c
d
a
b
c
20
40
SECONDS
POSTGANGLIONIC SPIKE ~ ARBITRARY UNITS

Fig. 18

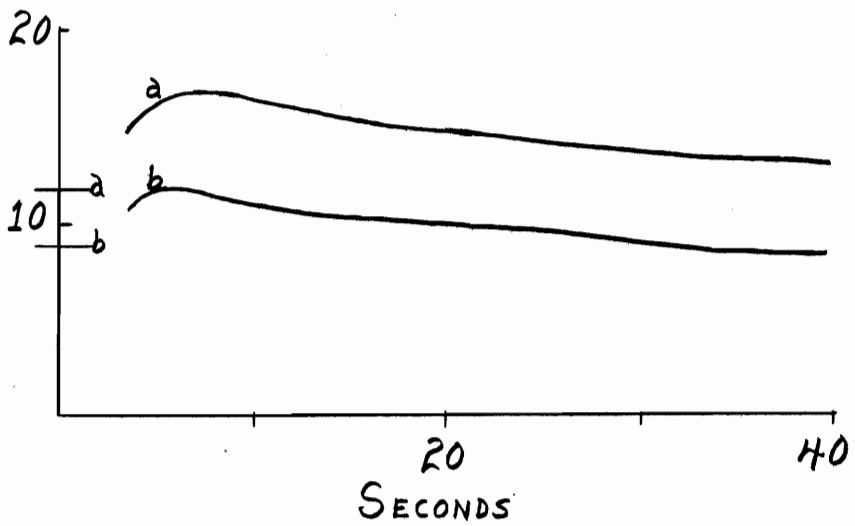
20
10
a
b
a
b
20
40
SECONDS

Fig. 19

Fig. 20. Records showing effect of Dilantin on ganglionic PTP.

Experiment G-18; curves a and d of figure 19 are drawn from these records. Records were obtained by an ink-writing oscillograph (Brush, BL-202) driven by a DC power amplifier (Brush, BL-962). Responses were monitored oscillographically. A slight change in the recording conditions causes the EKG to appear in lower record but not in the upper one.

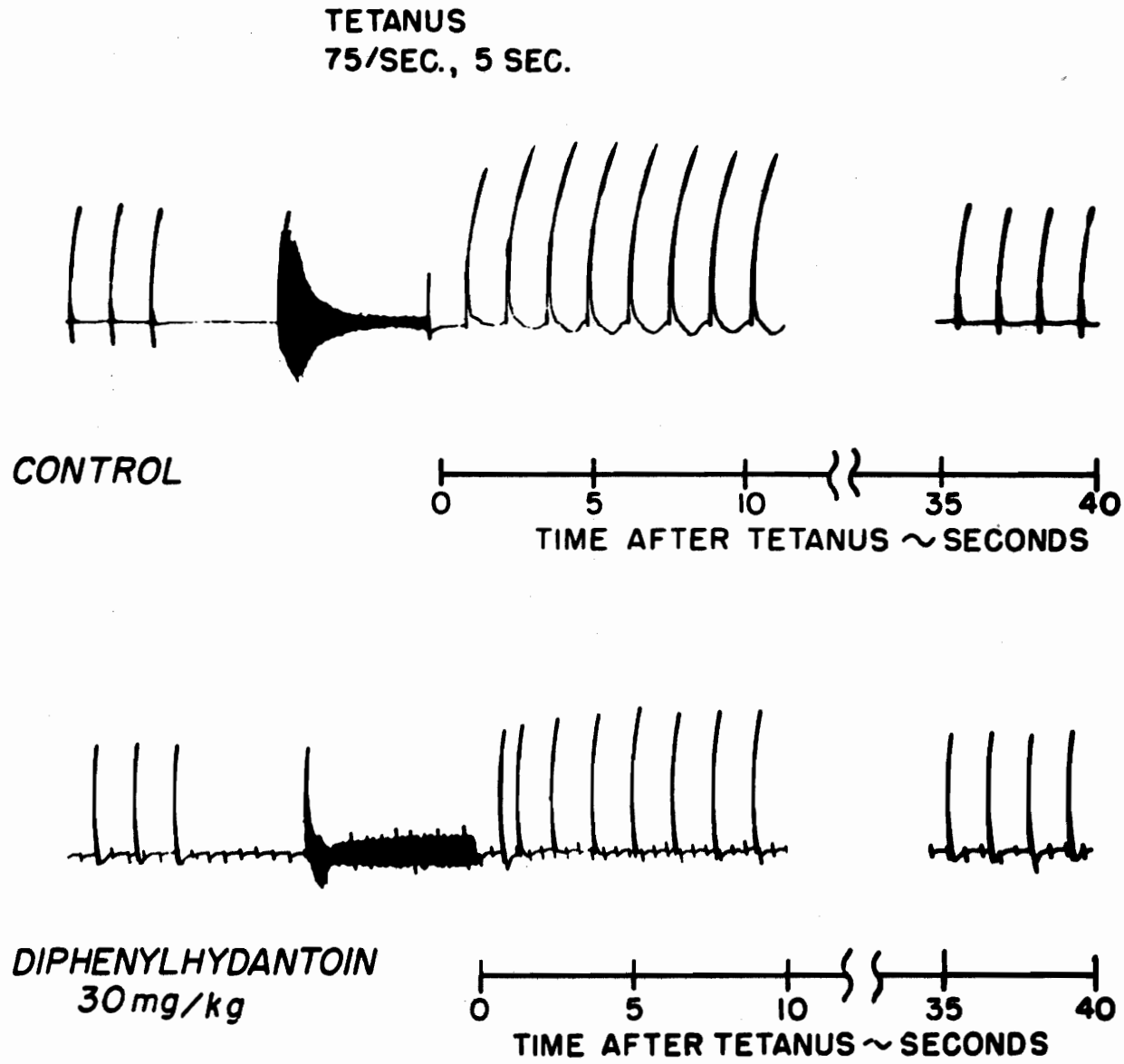
TETANUS
75/SEC., 5 SEC.
CONTROL
0
5
10
35
40
TIME AFTER TETANUS ~ SECONDS
DIPHENYLHYDANTOIN
30 mg/kg
0
5
10
35
40
TIME AFTER TETANUS ~ SECONDS

Fig. 20

EFFECTS OF DIPHENYLHYDANTOIN ON SYNAPTIC TRANSMISSION

by

Don W. Esplin

An abstract of a thesis submitted to the faculty of the University of Utah in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Approved by the faculty committee in

June, 1955

Herbert L. Borison, Chairman, Supervisory Committee

Department of Pharmacology

1955

The effects of diphenylhydantoin (Dilantin) on impulse transmission in monosynaptic and polysynaptic pathways of the spinal cord and in the stellate ganglion have been investigated. Unanesthetized spinal cats were used in all experiments and conventional electrical recording technics employed. In spinal cord experiments, 'square wave' stimuli were applied to dorsal roots or to individual hindlimb muscle- or cutaneous-nerves, severed distally. All relevant ventral roots were cut intradurally and the efferent impulses were recorded from a ventral root. In stellate ganglion experiments, stimuli were applied to the sympathetic chain immediately below the ganglion and efferent impulses were recorded from the cut inferior cardiac nerve. The amplified electrical responses were displayed on parallel dual-beam cathode-ray oscillographs and photographed. Doses of Dilantin between the minimally effective anticonvulsant and the minimally neurotoxic dose for cats (10 to 40 mgm./kgm.) were administered by slow intravenous infusion.

Dilantin was observed to have little effect upon the transmission of isolated impulses in monosynaptic pathways of the spinal cord or stellate ganglion but did reduce activity in spinal cord polysynaptic pathways. No effect of the drug upon facilitation has been observed in either type of experiment; primary inhibition has not been thoroughly studied.

The principal effects of Dilantin on transmission in monosynaptic pathways of the spinal cord and in the stellate ganglion are qualitatively identical. Dilantin deepens depression following transmission of a single impulse and hastens fatigue during repetitive stimulation. Post-tetanic potentiation, which follows repetitive synaptic activation, is dramatically

reduced by Dilantin. Evidence is presented to suggest that Dilantin decreases post-tetanic potentiation by limiting the reduction in synaptic threshold produced by tetanic stimulation. The significance of the effects of Dilantin on synaptic transmission are discussed in relation to the anti-convulsant action of the drug.